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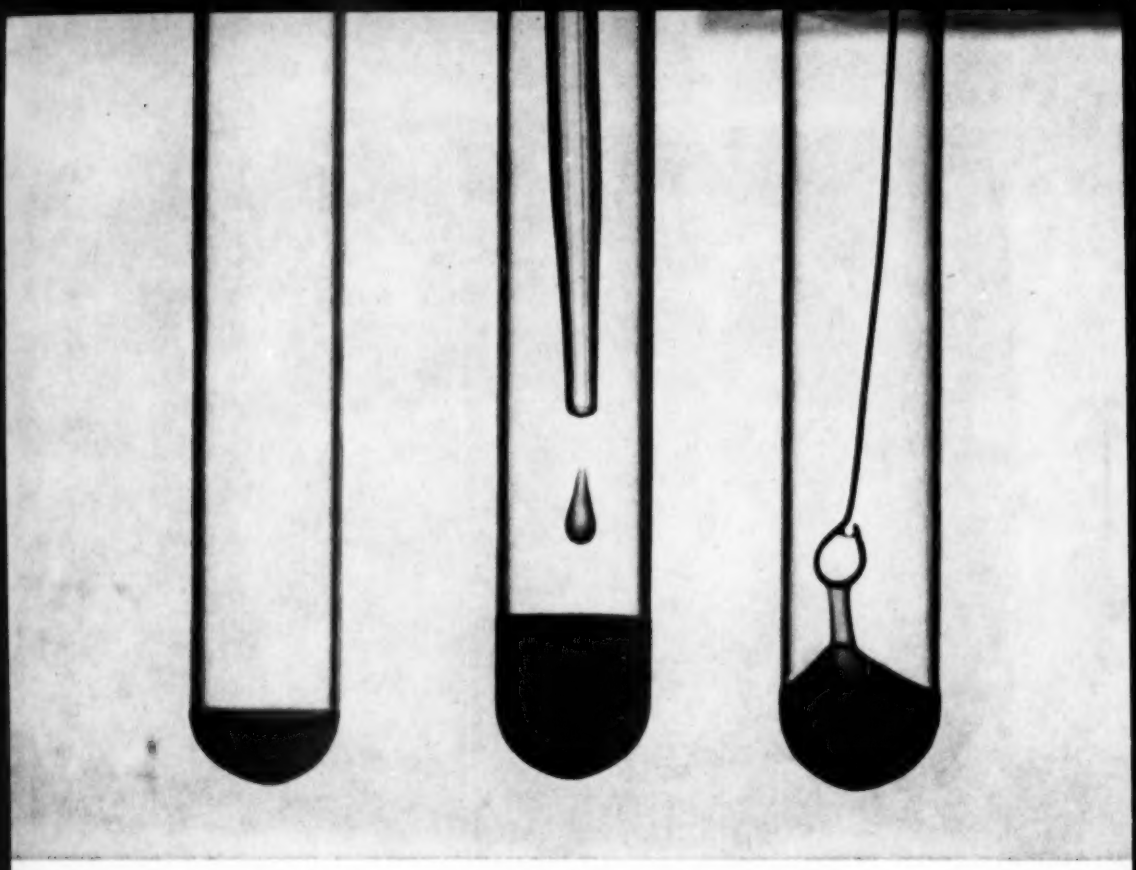

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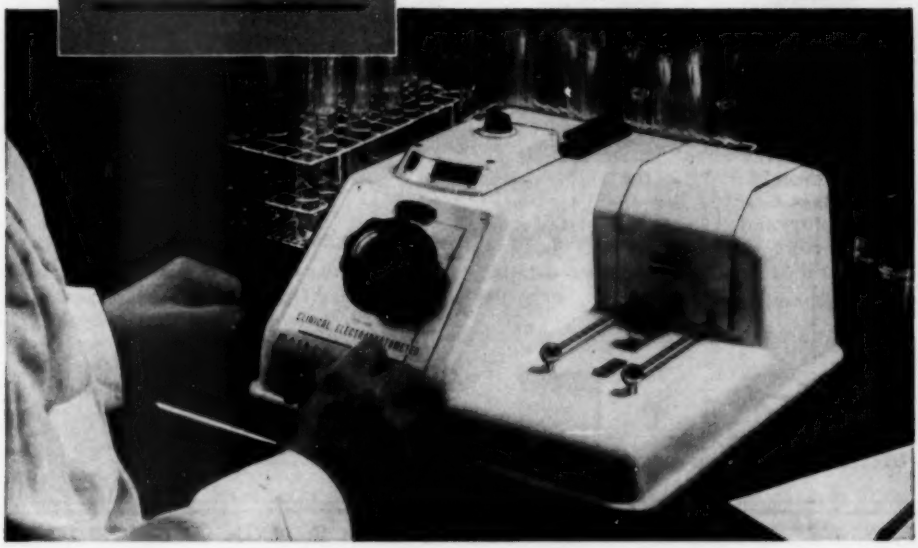

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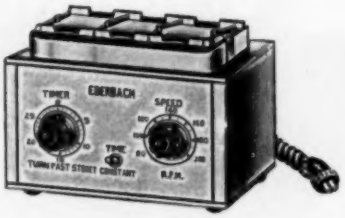

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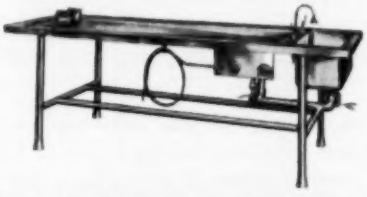

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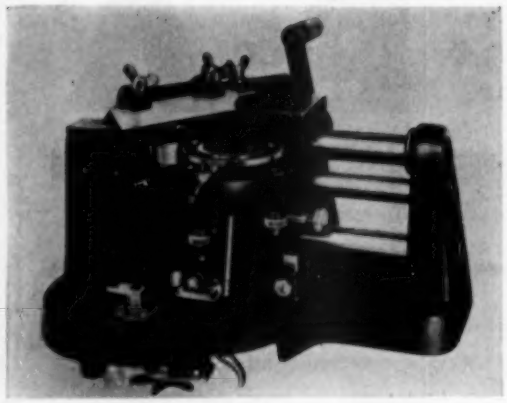

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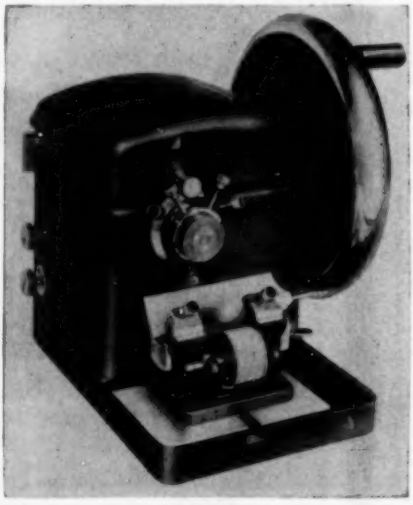

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DYNAMICS OF INFLAMMATION AND OF REPAIR

V. The Phenomena of Leucocytosis and Fever

VIRGIL H. MOON, M.D.
AND
GEORGE A. TERSHAKOVEC, M.D.
CORAL GABLES, FLA.

LEUCOCYTOSIS and fever are recognized features of local inflammation. There is general agreement (Ebbecke,* Arey,[3] Lewis,[4] Menkin,† Moon ‡) that the local response to injury is initiated not by the injurious agent, as Virchow believed, but by substances set free by the injured cells. We have reported experiments which corroborate that explanation. Menkin went further and postulated that the substance is one and the same regardless of the tissue injured and that it is a chemical entity. The evidence supporting this view has not been fully substantiated and accepted, but it is agreed that the stimulus which initiates inflammation is derived from the injured cells.

The occurrence of leucocytosis and increased temperature is not limited to local areas of injury. They occur as systemic reactions when injuries have been severe or extensive. Often these phenomena are independent of infections. Wilson [10] and his associates reported a leucocytic count of 38,000 to 45,000 within one hour after burns. In the early toxemic stage, hyperpyrexia was persistent and progressive. In these cases no infection had developed. In later stages of severe shock, the rectal temperatures were subnormal regularly. Harkins [11] cited numerous reports of leucocytic counts ranging from 20,000 to 50,000 shortly after extensive burns. He stated that hyperpyrexia is a characteristic feature in such cases. Cannon [12] had observed high leucocytosis in severely wounded men; this occurred too early and subsided too rapidly to be attributed to infection. Surgeons are familiar with moderate degrees of leucocytosis and fever after uninfected injuries and operations. "Fever accompanies sterile trauma." [13] These systemic reactions to extensive uninfected injuries have not been fully explained. Are they related to the same mechanism as the local reactions? Experiments were devised to produce evidence, if possible, bearing on this question.

METHODS

Two series of experiments were made, one on the effects of thermal injuries and another on the responses to intravenous injections of extracts of various tissues and of protein-split products. Rabbits were used throughout these experiments. Temperature readings and leuco-

The authors acknowledge the assistance of Maria A. Tershakovec.

From the Medical Research Unit, University of Miami, and the Pratt Veterans Hospital.

* References 1 and 2.

† References 5, 6, and 7.

‡ References 8 and 9.

cytic counts were made during several days to establish the norm for each animal; no rabbit was used if these examinations or the animal's behavior indicated any abnormality. The fur over the shoulders, back, sides, and hips was sheared closely with clippers. The rabbit was anesthetized deeply with ether; then the areas mentioned were immersed for five seconds in water at 88 to 95 C. Subsequent pain was prevented by injections of morphine. The scalded areas were estimated as about one-third of the total body surface. Temperature readings and blood counts were recorded at intervals during 7 hours and again at 24 hours, after which the animal was anesthetized and killed. After the same preparations, similar areas were frozen by immersion in a freezing solution of 95% alcohol and solidified CO_2 (dry ice) for 60 seconds at a temperature of —50 to —60 C. Anesthesia, tests, and treatment were the same as for injury by heat.

Tissue extracts were prepared as follows: muscle, liver, kidney, and lung were taken from a freshly killed rabbit. A weighed quantity of each tissue was suspended finely in 4 vol. of 0.9% solution of NaCl by means of a Waring Blendor. The mixture was then clarified by centrifugation at high speed; the supernatant fluid was adjusted to pH 7, sterilized by Seitz filtration, and stored in refrigeration until used.

A peptic digest of fibrin was prepared as follows: 5 gm. of fibrin (Nutritional Biochemical Corporation) was suspended in 100 ml. of H_2O and acidified with 5 N HCl to pH 2. Five hundred milligrams of pepsin (1:10,000, Nutritional Biochemical Corporation) was dissolved in a few milliliters of 0.1 N HCl and added to the fibrin suspension. The digestion mixture was incubated in a water bath at 37 C. for two hours. During incubation the reaction was maintained at pH 2. After incubation the digest was neutralized to pH 7, deproteinized by boiling for five minutes, centrifuged, and filtered. This digest contained 5 gm. of dry residue in 100 ml. Several commercial products of protein digestion, including Bactoprotone, Neopeptone, Proteose-peptone (each by Difco), and Witte's peptone were tested. A solution of each product mentioned was made in 0.9% solution of NaCl; the solutions were adjusted to pH 7, centrifuged for clearing, sterilized by Seitz filtration, and stored in refrigeration. These substances contained products of protein cleavage whose exact chemical composition is not known. Such products also are formed by autolysis of normal tissues.

RESULTS

Leucocytes.—Injury by heat was followed by a sharp progressive rise in the leucocytic count, which reached its maximum four hours after the injury. Freezing caused a slight decline in the leucocytic count during the first hour, after which the count rose, reaching its peak four hours after freezing. The average leucocytic count for each group is shown graphically in Figure 1.

It is seen that the average leucocytosis after freezing was less than that after scalding, perhaps because freezing produced a less severe injury to the tissues. The average count in each group declined in 24 hours almost to the preinjury level.

Temperature.—An unexpected decline in temperature occurred in each animal after injury by heat. This was severe, ranging from 3 to 6 degrees F., and was sustained during several hours. The maximum decline occurred between three and six hours after the burn. This result is opposite to observations of increased temperature after burns in man. Perhaps the low temperatures recorded were due to shock resulting from the burns. Counts of erythrocytes in each rabbit were sharply increased immediately after the injury, indicating hemoconcentration. This ranged between 32 and 45% two hours after the burn, and it remained at somewhat lower degrees throughout the day. Decreased circulation and temperature, due to shock, may have counteracted any pyrogenic effects the injury may have caused.

There was a similar decline in the temperatures after freezing; moderate hemoconcentration developed also. However, in this group of animals the severe freezing was a factor which may have lowered the temperature for a time. There remains

the possibility that the responses of rabbits and of man to pyrogenic agents differ somewhat. However, rabbits are susceptible to a wide variety of pyrogens and are used practically in testing solutions for pyrogenic effects.

Effects of Tissue Extracts and of Protein-Cleavage Products.—Each of 12 rabbits received by intravenous injection 10 ml. of extract of rabbit muscle prepared as described under "Methods." Temperature readings and leucocytic counts were made before and at intervals after the injections. The temperature increased by 1 to 4.8 degrees F. in 11 animals; the average increase was 2.0 degrees. In one rabbit the temperature decreased by 1.3 degrees three hours after the injection. In eight rabbits the leucocytic count decreased by about 50% shortly after the injection. In four of these, the leucopenia was transient and was followed immediately by leucocytosis; in four, leucopenia continued during the day but leucocytic counts ranging

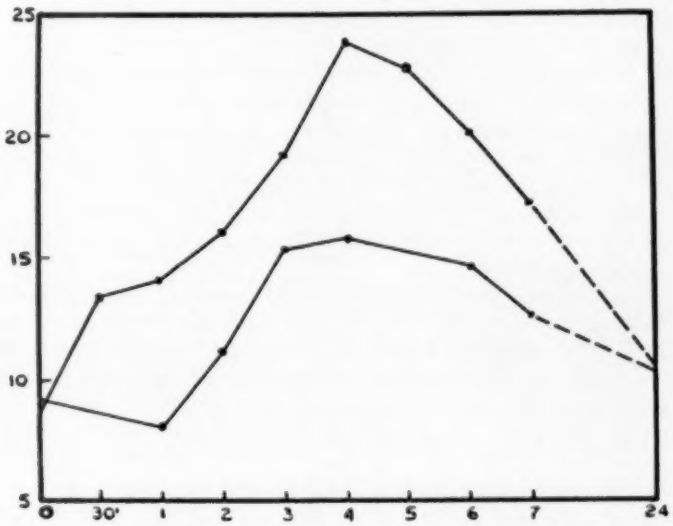

Fig. 1.—Leucocytosis after thermal injuries. Leucocytic counts in thousands are shown at left; time in hours, at bottom. Upper graph is composite curve of leucocytic counts in 10 rabbits after injury by heat. Lower graph is curve of leucocytic counts in 10 rabbits after freezing.

from 10,300 to 23,000 were found some 16 hours later. In the remaining four, the leucocytosis was immediate, without a preceding interval of leucopenia. A moderate increase in temperature developed in 11 out of 12 rabbits after the intravenous injection of muscle extract. In all rabbits moderate to severe leucocytosis developed; in eight this was delayed and was preceded by leucopenia.

In some particulars, extract of lung produced severer effects than that of muscle prepared in the same manner. In three rabbits, the intravenous injection of 1.0 ml. of lung extract resulted in moderate leucopenia followed by leucocyte counts of 18,200, 27,500, and 36,800, respectively, in three hours. Simultaneously, the temperature declined by 0.9, 1.0, and 2.1 degrees F., respectively. In two rabbits, the injection of 5.0 ml. caused immediate death. Necropsy revealed acute pulmonary and hepatic hyperemia and distention of the right heart. Two other rabbits receiving

5.0 ml. responded with a rise of 1.1 and 2.0 degrees in temperature and leucocyte counts of 22,650 and 17,150, respectively. Extracts of liver and kidney had effects like those of lung but less in degree. Injections of 5 and 10 ml. caused a rise of 1.0 to 2.5 degrees in temperature and leucopenia of about 40% followed by varying degrees of leucocytosis. Six rabbits, as controls, received 10 ml. of 0.9% solution of NaCl intravenously. No significant variations in temperature or in leucocytic counts followed these injections.

We have no evidence as to why extract of lung caused severer reactions than either of the other tissues tested. The immediate death and the necropsy findings remind one of the effects of histamine given intravenously. Also, one remembers that lung substance has a higher content of histamine than other tissues. However, the dose, 5 ml. of the solution which caused immediate death in two rabbits, contained the soluble substance from 1.0 gm. of lung tissue, or approximately that from 0.2 gm. dried weight of lung. It seems questionable that this minute amount of lung would contain a fatal dose of histamine.

Several products of protein digestion prepared as described under "Methods" were tested in the same manner as were the extracts of tissues. Each of four rabbits received 10 ml. of a 2% solution of Bactoprotone by intravenous injection. In each the temperature increased by 1.9 to 3.6 degrees F. in from one to three hours; the average increase was 2.5 degrees. Leucopenia developed within 30 minutes after each injection; the average decrease was 1,380 cells. The maximum leucocytosis occurred at two and three hours after the injection, at which time the average leucocytic count was 17,330.

Each of four rabbits received 10 ml. of a 4% solution of Neopeptone intravenously. The reactions were less marked than from Bactoprotone. The temperature increase ranged from 0.9 to 2.5 degrees F., averaging 1.6 degrees. Leucopenia with an average decrease of 2,280 cells occurred within 30 to 60 minutes and was followed by a moderate leucocytosis in each animal, the count averaging 12,850.

A peptic digest of fibrin was prepared as described under "Methods." The effect of this was tested on five rabbits by intravenous injection. The first injection, 10 ml., was followed by a decrease of 5,850 in the leucocyte count within one hour. The count remained subnormal throughout the day, but had risen to 14,100 the next morning—an increase of 5,650 above the preinjection level. The temperature rose 2.5 degrees F. in one hour and reached its maximum, a 5.1 degree increase, three hours after injection. The temperature declined slowly and was 1.8 degrees F. above the preinjection level 24 hours later. Moderate hemoconcentration developed, and it was difficult to secure blood from peripheral vessels—indicating shock. Death occurred about 36 hours after injection.

Necropsy showed severe pulmonary and hepatic hyperemia and dilatation and congestion of the right heart. Microscopic examination showed pulmonary hyperemia and edema; the hepatic sinusoids were distended with blood and the hepatic cells were granular and vacuolated. Small local areas of necrosis were seen in various parts of the lobules (Fig. 2). The renal tube epithelium was granular, vacuolated, and, in some areas, necrotic. In many tubules, the cells had desquamated into the lumina (Fig. 3). These changes were severest in the proximal convoluted tubules. The features described are characteristic findings after death from secondary shock.[14] They are attributed not to the effects of any specific toxic substance, but to lack of oxygen.

Three other rabbits were given the same dosage, 10 ml. of peptic digest intravenously. This caused an increased temperature of 4.1, 3.9, and 4.1 degrees F., respectively, and leucopenia, with an average decline of 5,983 cells in three hours. In seven hours, severe leucocytosis developed, with an average increase of 20,550 cells above the preinjection level; this persisted at lower levels on the following day, but each rabbit lived.

Each of three rabbits received 5 ml. of the same digest intravenously. The temperature of one rose promptly, and the leucocyte count declined by 2,950 cells. An hour later the temperature fell 2.8 degrees F. below the preinjection level, and circulatory inefficiency made it impossible to get samples of blood from peripheral vessels. Death occurred some hours later, and the necropsy findings were identical with those in the preceding experiment. In the two other rabbits which received

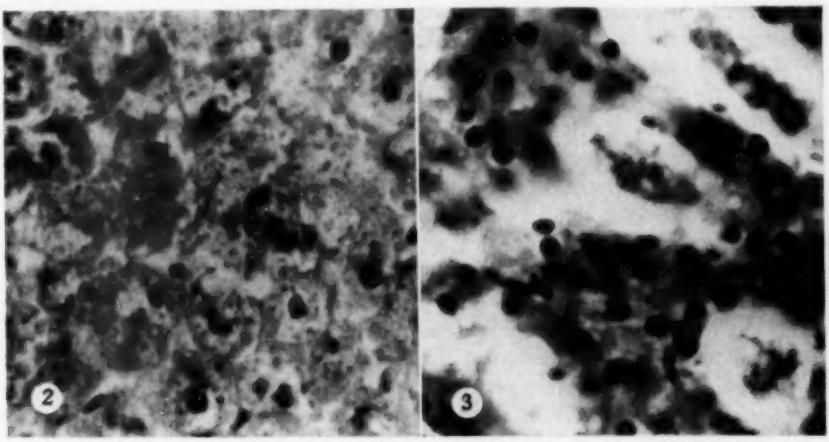

Fig. 2.—Photomicrograph of liver after death from peptic digest of fibrin given intravenously. Granular degeneration, vacuolization, necrosis, and disintegration of cells are seen; × 300.

Fig. 3.—Photomicrograph of kidney from rabbit of Figure 2. Vacuolization, necrosis, and desquamation of tubular cells are seen; × 300.

the same dose, the temperature rose 3.4 and 2.0 degrees F. and the leucocyte count declined 4,300 and 5,700 cells, respectively, in three hours. This decline was followed by moderate leucocytosis; these animals recovered.

It is known that Witte's peptone, a commercial digest of fibrin, is inconstant in composition and properties; samples from different batches may vary widely in their effects. We tested a sample for comparison with other products of protein cleavage. Each of four rabbits received 10 ml. of a 10% solution by intravenous injection. In each, the temperature rose promptly, reaching its maximum in three hours, and a gradual decline followed; the average rise was 3.1 degrees F. A prompt leucopenia developed, with an average decline of 3,812 cells in leucocytic count. This was followed by moderate leucocytosis which did not subside during 24 hours. Each of these rabbits recovered. The effects of this sample of Witte's peptone were less severe than the peptic digest of fibrin made in this laboratory.

A more extended series of experiments would have been made except that these would have established no new facts or principles. Our results are sufficient to emphasize and to corroborate observations made years ago upon the effects of tissue extracts and other protein derivatives, as described hereafter.

GENERAL REACTIONS TO NITROGENOUS SUBSTANCES

The reactions to burns, freezing, and injections of tissue extracts and of protein derivatives appear related to nonspecific reactions evoked by a varied assortment of protein-containing substances of diverse origins. These include many bacteria, pathogenic and otherwise, and products derived from them; blood and sera, both normal and immune; exudates, such as blister fluid, pleural and ascitic fluids, and cerebrospinal fluid; proteins, including egg albumen, serum albumin, nucleoproteins, fat-free milk, casein, zein, and gelatin; proteoses, peptones, polypeptides, and other products of protein cleavage, and extracts of leucocytes, of various tumors, and of cartilage, spleen, and other normal organs and tissues (see Petersen [15] for review).

Some 40 years ago the parenteral use of the substances mentioned, including vaccines and sundry bacterial products, was in vogue for therapeutic purposes. Medical literature of that period contains a wealth of clinical and experimental observations on the effects of such agents. These had one property in common: When given parenterally, in adequate dosage, they evoked a "general systemic reaction" consisting of chills, sweating, a temperature ranging from 101 to 104 F., rapid pulse, and a leucocytic count of 20,000 to 40,000. The severer reactions included vomiting, low blood pressure, and other signs of shock; death sometimes resulted. The therapeutic aim was to evoke a mild or moderate reaction, which often was followed by clinical improvement. Physicians of that period lacked an understanding of the mechanism of these effects, which now are identified with the "alarm reaction." Injury or stress of various types evokes responses which are protective or defensive in nature. These include the discharge of hormones from the pituitary and adrenal glands, followed by leucocytosis, increased temperature, and other features. It is understandable why mild reactions of this kind were sometimes beneficial clinically.

Vaughan [16] derived a "crude soluble poison" by hydrolytic cleavage of various proteins: egg albumen, serum albumin, casein, edestin, zein, and the cellular substance of 10 common species of bacteria. Minute amounts of this poison given parenterally caused severe systemic effects, including fever and leucocytosis. Larger amounts caused shock-like manifestations and death. This substance appeared to be a primary cleavage product; it responded to the biuret and Millon's tests, but the authors were unable to determine accurately its chemical structure.

The effects of Vaughan's poison and of other protein derivatives varied widely, depending upon the amount given. A small dose would cause only moderate leucocytosis and slight fever; a larger dose might cause leucopenia followed by leucocytosis, while a fatal dose might cause this same reaction or might cause a decline in temperature, in blood pressure, and in the leucocytic count, followed by death. Vaughan reported producing these and other features by varying the intervals and the dosage of simple proteins (egg albumen, serum albumin, zein). Years ago, Zinnser [17] reported that intoxicating doses of various protein-containing substances

caused decreased temperature and leucopenia, while minute doses of the same substances caused fever and leucocytosis. The variations in some of our results coincide with these findings.

Nonspecific protein therapy was superseded by more reliable methods, but it left a heritage of clinical and experimental data which aid in the interpretation of systemic reactions. The numerous agents which will cause fever and leucocytosis include endogenous, or native, as well as foreign proteins. A small local burn of the skin will cause prompt immigration of leucocytes into the area and a local rise in the temperature. An extensive burn will cause immediately a high leucocytic count—30,000 to 50,000—and pyrexia. In the former, the reactions are attributed to substances released locally from the damaged cells; in the latter, one suspects that the same substances released in large amount produce the same features systemically as part of the "alarm reaction." In view of the number and diversity of substances which will produce the effects mentioned, it seems illogical to attribute the effects severally to individual chemical entities.

Menkin § has made numerous reports dealing with the effects of substances derived from purulent exudates. These contained products of the autolysis of leucocytes and of the digestion of proteins by leucocytic enzymes. From this substrate several substances were separated by precipitation, centrifugation, and dialyzation. When tested on animals, these products caused local inflammation with capillary permeability and immigration of leucocytes; systemic effects were fever, leucopenia, leucocytosis, and sometimes necrosis of cells in parenchymatous organs. These substances were designated leucotaxine, pyrexin, leucopenin, LPF (leucocytosis-promoting factor), and necrosin, respectively, and were believed to be chemical entities. Necrosin was identified with the euglobulin fraction; others were regarded as polypeptides. However, the assumption that these substances derived from purulent exudates are identical with various protein-cleavage products resulting from injury to tissues appears inadequately supported by the evidence.

One of these substances, "necrosin," may be cited as an example. It was obtained by precipitating purulent exudate by one-third saturation with $(NH_4)_2SO_4$, treating the precipitate with H_2O, then dialyzing the solution and obtaining an insoluble precipitate, "necrosin," which had enzymatic properties. This product was highly toxic; subcutaneous injections of 1 ml. caused necrosis and intense inflammation. The intravenous injection of 10 ml. in dogs caused high fever and severe leucopenia followed by leucocytosis. Vomiting, bloody diarrhea, and listlessness developed, followed by death within 24 hours. Microscopic examination showed severe degeneration and necrosis of hepatic and renal cells. The author recognized these features as indicating shock. Degenerative and necrotic changes in the liver and kidney are characteristic items in the pathology of shock.[14] They usually are attributed to anoxia rather than to toxic substances. Since "necrosin" causes fever, leucopenia, and leucocytosis, its differentiation from pyrexin, leucopenin, and LPF appears uncertain; that the substance in question evoked a strong alarm reaction seems evident.

Should Menkin's interpretations be accepted, then the numerous and various substances which will cause a nonspecific protein reaction would be assumed to contain each of his hypothetical substances, as would the extracts of normal tissues and

§ References 5, 6, and 7.

the products of protein digestion used in our experiments. Some of these caused reactions indistinguishable from those of "necrosis," including local necrosis in the liver and kidney (Figs. 2 and 3). Yet none of the protein-cleavage products tested by us contained euglobulin or other whole proteins.

An alternate interpretation integrates smoothly with the evidence at hand: The various substances under consideration will cause an acute inflammatory reaction when injected locally [9] and will call forth varying degrees of the alarm reaction when introduced into the blood stream. Minor effects include variations in temperature and in leucocytic counts; major effects may include manifestations of shock accompanied by degeneration and/or necrosis in parenchymatous organs. It is uncertain whether the variations in temperature and in leucocytic counts were the direct effects of the substances upon the heat-regulatory center and bone marrow, respectively, or whether those effects were mediated through the endocrine system.

Animal organisms possess physiological mechanisms which are activated by injury or stress. One of these—the inflammatory reaction—is a local defensive and reparative process apparently triggered by substances released from injured cells; these cause local permeability of capillaries and attract leucocytes into the injured area. When injury has been severe or extensive, the immediate reaction is systemic as well as local and includes leucocytosis and fever. Our results suggest that the systemic reaction also is a response to substances absorbed from damaged tissues. Such substances may be the unchanged cytoplasm of the cells or, more probably, products of autolysis. These mechanisms respond both to extracts of normal tissues and to products of protein cleavage. The local injection of these evokes an acute inflammatory reaction.‖ The intravenous introduction of the same substances causes a systemic reaction, including fever and leucocytosis, which resembles the systemic response to extensive uninfected injuries.

The degradation of proteins can be accomplished by either hydrolytic or enzymatic agents, yielding split products ranging from complex proteoses to amino acids. Neither the chemists' skill nor the living cells in various tissues can differentiate the origins of these cleavage products. It appears that neither the endothelium, the heat-regulatory center, the bone marrow, nor the endocrine glands have the ability to distinguish cleavage products of tissue proteins from those of other proteins. As a result, several physiologic reactions respond similarly to such products regardless of their origins.

SUMMARY

Extensive injuries by heat or cold cause a sharp rise in the leucocytic count in the circulating blood. In rabbits, these injuries are followed by hypothermia, whereas hyperthermia occurs after burns in man.

Variations in temperature and in leucocytic counts followed the intravenous injection of watery extracts of normal tissues. A rise in temperature occurred with few exceptions; in some instances this was preceded by a transient decline. Moderate to severe leucocytosis followed the injections; usually this was preceded by varying periods of leucopenia.

Similar variations in temperature and leucocytic counts followed injections of several products of protein digestion. In some instances shock was apparent and

‖ References 9 and 18.

in a few cases was fatal. Necropsy findings included visceral hyperemia, severe degeneration, and necrosis in the liver and kidneys.

Our findings coincide with those of earlier workers who noted that nonspecific protein reactions vary with the dosage. It appears that sundry products derived from animal tissues will excite both local and systemic reactions and that the resulting fever, leucopenia, and leucocytosis are effects of the parenteral introduction of proteins and their derivatives rather than of hypothetical chemical entities.

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SMALL PANCREATIC ISLET ADENOMATA IN ADDISON'S DISEASE

Report of Two Cases with an Analysis of Islet Size in These and Fourteen Other Cases

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DETAILED investigations have been made of the disorder of carbohydrate metabolism encountered in Addison's disease.* It has been shown that the blood-sugar level is usually low-normal and sometimes below 0.050 gm. per 100 cc.,† that the oral and intravenous sugar tolerance tests are abnormal, and that there is a greatly increased sensitivity to insulin, abolished by the so-called sugar-active corticoids. These abnormalities have been explained in terms of a diminution in hepatic gluconeogenesis and an increased utilization of muscle glycogen. The latter have been attributed to adrenocortical hypofunction, and little attention has been paid to the possibility of an overproduction of insulin by the pancreas, a theory recently advanced by Hinerman,[5] on the grounds that there is in every case gross hyperplasia of islet tissue. This claim has serious clinical and therapeutic implications. With a view to testing it, a histological examination has been made of the pancreas in 17 of 37 necropsies performed on patients with Addison's disease in this institute. In two of these cases small islet adenomata were found. The criteria for the diagnosis of Addison's disease are stated elsewhere.[6]

METHODS

In 15 instances, following routine practice in this institute, the pancreas was dissected free and weighed. Sections were taken for microscopic examination in 17 cases; in 8 from the head, body, and tail of the pancreas, in 2 from the body and head and body and tail, in 5 from the body alone, and in 2 from unspecified sites. In Case 1 the fixative was Müller's formol and in the remainder formalin. The various islet cells were differentiated by two of Gomori's techniques, using chrome-hematoxylin [7] and aldehyde-fuchsin.[8] The latter was much the more successful and was used in every case.

An approximate estimation of the amount of islet tissue was obtained in eight instances from a knowledge of the weight of the pancreas and from estimation of the proportion of islet and acinar tissue in transverse sections from the head, body, and tail according to the methods of Heiberg [9] and Ogilvie.[10] Fifteen fields from each section, namely every 10th in each row, were drawn with a camera lucida using a ⅔ in. (16.9 mm.) objective, and areas were estimated with a planimeter accord-

From the Bernard Baron Institute of Pathology, The London Hospital.

* References 1 and 2.

† References 3 and 4.

ing to the method of Susman.[11] A figure for total islet "weight" was obtained by making a number of unjustified assumptions; for example, that these sections were representative of the gland as a whole and that such factors as the differential shrinkage of parenchyma and interstitial tissue, the varying specific gravities of these components, and the varying rate of autolysis in different corpses could all be neglected.

For the purposes of comparison, Hinerman's technique, which suffers from far greater shortcomings, was applied in 16 instances; 35 consecutive islets were measured in their longest diameter with a micrometer, using a ⅙ in. (4.2 mm.) objective; the islets or part islets containing B granules (staining with aldehyde-fuchsin) in 236 consecutive fields (0.2525 sq. cm.) were counted. Hinerman counted the number of islets containing B granules (staining with chrome-hematoxylin) in an area 0.6 cm. in diameter (0.2826 sq. cm.) in unspecified parts of the pancreas. He did not state the weight of the pancreas or patient, and in two cases the extent to which islet size might have been increased by amyloidosis was not mentioned.

His method substitutes a measurement of one dimension for the two measured by Ogilvie. It does not specifically take account of the fact that islets are held to increase in number towards the tail of the human pancreas,[12] and it takes little account of the gross variations so commonly encountered in the weight and adiposity of the normal gland. Hinerman, nevertheless, claimed that this technique was adequate for the recognition of gross variations in the amount of islet tissue. In this respect it scarcely merits comparison with Ogilvie's method. He also claimed that any apparent increase in islet number or size indicated an absolute increase in the amount of islet tissue. This claim was largely based on Tejning's [12] investigations of the rat pancreas. There is little to recommend this transference to the human of deductions derived from a study of the rat.

CLINICAL FINDINGS

In none of the 17 patients with Addison's disease were there any symptoms which could necessarily be identified with abnormal carbohydrate metabolism, with the exception of Case 3, already reported by Simpson,[13] where death occurred in coma. This patient on admission had a blood-sugar level of 0.05 gm. per 100 cc. Two months later, and during intravenous treatment with cortical extract, he developed glycosuria and hyperglycemia (sugar levels up to 0.36 gm. per 100 cc.). Death followed a day and a half after the administration of 20 units of insulin; this had caused a severe hypoglycemia.

In two other cases (5 and 13) levels of 0.050 and 0.067 gm. per 100 cc. were recorded. In one patient (Case 6) with a small islet adenoma the lowest level was 0.077 gm., rising to 0.144 gm. after the administration of 50 gm of glucose. In Cases 2, 8, 11, 14, and 16, the lowest values varied between 0.08 and 0.087 gm. per 100 cc.

PATHOLOGICAL FINDINGS

Seven glands were of somewhat reduced weight (that is, below 57.7 gm., the lowest weight recorded in our series [Table 2] of accidental deaths of the appropriate age).

Histological examination revealed a normal proportion of B granules in all save Cases 1 and 3, in which these granules were entirely lacking. In the first, after Müller formol fixation, the islets seemed in other respects normal. In Case 3 islet cell cytoplasm was very scanty, and there was a focal diffuse excess of

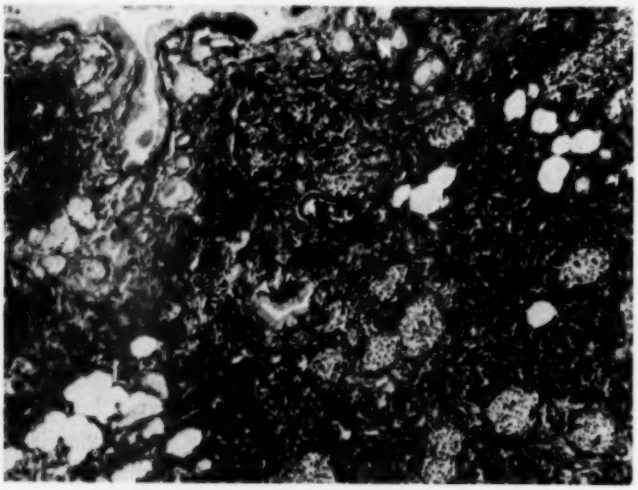

Fig. 1 (Case 5).—Local aggregation of islets in section from tail of pancreas. Mallory's azan stain; × 61.

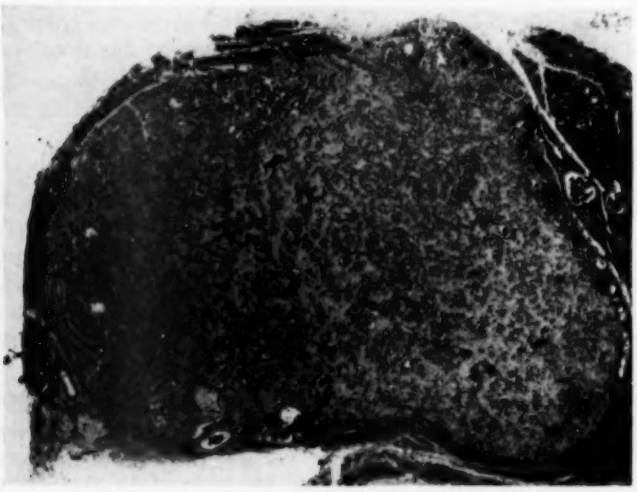

Fig. 2 (Case 8).—Islet adenoma. Van Gieson stain; × 16.

interstitial tissue. Focal lymphocytic infiltration was noted in this gland and also, to a lesser degree, in Cases 4 and 10. In Case 5 a section from the tail of the pancreas showed a local aggregation of islets, some periductal but the majority lying between pancreatic acini (Fig. 1). Around the adjacent ducts there was

an increase in fibrous tissue. The pancreas was of low-normal weight (60.5 gm.). Postmortem degeneration was fairly marked in Cases 4 and 11 but did not prevent islet area measurements in the former.

Details of micrometric and planimetric estimations are recorded in Table 1, and these may be compared with figures derived from the examinations of glands from patients dying from unrelated diseases. In these cases and in Addison's disease the figures show considerable variation; they certainly do not indicate any gross excess of islet tissue in Addison's disease.

TABLE 1.—*Micrometric and Planimetric Analysis of Pancreatic Islet Tissue in Addison's Disease*

Case No.	Sex	Age	Weight of Pancreas		Micrometry *		Planimetry †	
			Gm.	Gm./Kg. Body Weight	No. Islets/ Sq. Cm.‡	Mean Longest Diameter of 35 Islets §	No. Islets/ Sq. Cm.‖	Percent Islet Area
Idiopathic								
1	F	51	.....	.....	135.6	137.5	.....	.....
2	F	47	45.8	1.132	120.2	84.2	.....	.....
3	M	16	15.7	0.606	132.0	80.2	193.0	3.196
4	M	43	60.7	1.021	73.8	120.6	195.5	1.458
5	F	34	60.5	1.622	124.2	98.1	160.5	1.296
6	F	26	54.3	1.308	206.0	109.2	371.0	2.796
7	F	51	66.8	1.230	431.0	107.1	240.5	3.820
8	F	38	.....	.....	143.5	145.0	.....	.....
9	F	38	55.9	1.782	155.5	150.8	182.5	1.500
Tuberculous								
10	M	29	56.6	1.112	151.0	103.3	.....	.....
11	F	48	68.2	0.994	Severe focal postmortem degeneration			
12	M	39	55.0	1.115	353.0	82.7	.....	.....
13	F	42	35.2	0.684	175.0	130.8	202.0	3.69
14	M	35	60.2	1.118	144.8	106.0	.....	.....
15	M	39	82.7	1.256	85.4	136.2	287.0	1.914
16	F	22	40.4	1.037	174.5	96.0	.....	.....
17	F	22	68.1	1.227	132.0	137.5	.....	.....
Controls								
1	M	15	76.7	1.562	101.0	112.8	170.0	1.56
2	F	20	72.8	1.758	101.0	100.7	169.0	2.09
3	M	36	78.6	1.556	278.8	108.9	.....	.....
4	M	42	76.1	1.900	379.0	72.9	.....	.....
5	F	54	115.8	1.915	202.0	105.9	.....	.....
6	M	33	74.8	1.030	89.3	180.2	275.0	3.5
7	F	64	86.2	1.220	178.5	101.4	.....	.....

* Hinerman.[5]
† Ogilvie [10] and Susman.[11]
‡ Section from body of pancreas.
§ In microns.
‖ Sections from head, body, and tail of pancreas.

ISLET ADENOMAS

In Case 8 a section from the body of the pancreas revealed a small islet adenoma measuring after fixation 0.8 by 0.45 cm. This was composed of forking trabeculae of cubical and polygonal cells, separated from each other by pale eosinophilic, picrophilic, somewhat acellular, connective tissue (Fig. 2). The adenoma cells did not contain B granules (as stained by chrome-hematoxylin or by aldehydefuchsin) and lacked the staining properties of A and D cells. On the other hand, in adjacent islets there were normal B cells (Fig. 3).

A second but smaller adenoma, measuring 0.13 cm. in diameter, was present in the body of the pancreas in Case 6 (Fig. 4). Histologically it was similar to that in Case 8, but it possessed a thicker fibrous capsule and no hyalinization of the stroma. Adjacent islets contained B granules.

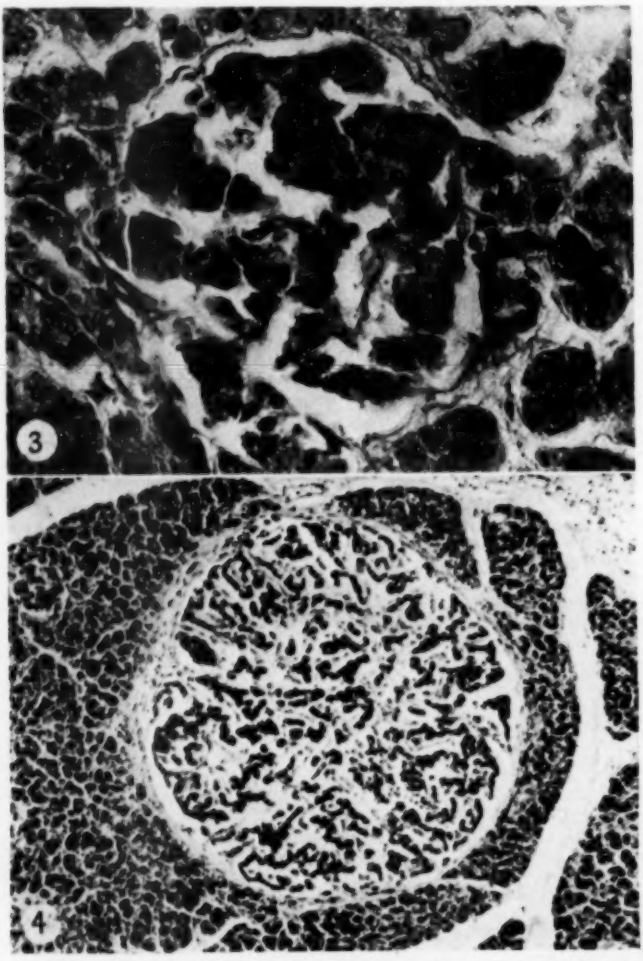

Fig. 3 (Case 8).—Normal islet adjacent to adenoma to show cells containing dark fuchsinophil B granules. Gomori's aldehyde-fuchsin stain; × 480.

Fig. 4 (Case 3).—Islet adenoma. Hematoxylin and eosin; × 89.

COMMENT

Apart from Hinerman's claim that there is invariably a great hyperplasia of islet tissue, little has been written about the pathology of the pancreas in Addison's disease. In particular, the occurrence of islet adenomata, observed in two of our cases, has been hitherto unsuspected. However, although their presence would con-

form well with the islet hyperplasia noted by Hinerman, we could find little to support the latter's claim. In evidence of this it will be shown, first, that the pancreas shows a tendency towards a reduction in weight in Addison's disease and, second, that there is no gross variation in the proportion of islet tissue in individual sections.

The Weight of the Pancreas.—An analysis of the weight of the pancreas must take into account the marked variation noted in the weight of the normal gland. Thus, although the average weight is said to be about 88 gm.,[14] our own figures, derived from an analysis of cases of accidental death occurring in the same decades of life as our cases of Addison's disease (15 to 59 years), show a range of between 57 and 123 gm. In Table 2 these figures are expressed as a percentage of body weight, and are compared with similar figures derived from Addison's disease and from cases of diabetes mellitus with pancreatic atrophy (the "ribbon" pancreas). It will be seen that in Addison's disease the percentage weight of the pancreas lies commonly between 1.1 and 1.229, that is in general below that of the normal gland,

TABLE 2.—*Comparison of Weight of Pancreas Expressed as a Percentage of Body Weight in Cases Dying from Addison's Disease, Diabetes Mellitus, and Accident*

Weight of Pancreas as Percentage of Body Weight	Accidental Death	Addison's Disease	Diabetes Mellitus with "Ribbon" Pancreas
0.500-0.699	0	1	4
0.700-0.899	0	0	3
0.900-1.099	2	3	3
1.100-1.299	9	10	0
1.300-1.499	10	1	3
1.500-1.699	7	1	0
1.700-1.899	4	1	0
1.900 on	2	0	0
Total number of cases	34	17	13
Weight of pancreas	57-123 gm.	15.7*-82.7 gm.	32.4-52 gm.

* In seven instances less than 57 gm.; in four instances less than 52 gm.

but above that of the "ribbon" pancreas. Analysis of the weight of the gland perhaps exaggerates this trend, for seven weighed less than the minimum weight of the normal gland in our series of accidental deaths, while four were within our diabetic range. It is of interest that in 19 cases of Simmond's disease reviewed by Sheehan and Summers [15] a similar range of weights was noted (20 to 100 gm., mean 46 gm.).

We cannot be certain that this trend towards atrophy is a specific feature of the disease. It is possible that this is the case, for nonspecific features which might lead to a reduction in pancreatic weight, such as obvious cachexia, or generalized tuberculosis, were only found in three instances (Cases 1, 3, and 10), while it was noted that in most cases (e. g., 4 to 9, 11, 14 to 17) the body seemed actually well-nourished when examined at necropsy. On the other hand, the percentage weight of only one gland (Case 3) was below our range of normals, and this was the only gland which seemed microscopically abnormal; that is, apart from the two containing islet adenomata.

The Evidence in Favor of Gross Islet Hyperplasia.—This tendency towards pancreatic atrophy has been noted previously at least in two cases of Addison's disease uncomplicated by diabetes mellitus,‡ and, indeed, Hinerman made the same

‡ References 16 and 17.

observation, but without providing any figures. Consequently, if, as he claims, there is an increase in the amount of islet tissue, this increase as judged from the study of individual sections should be obvious. This, using his own technique (Table 3), we failed to confirm.

It will be seen that in our series (Table 3) there was a considerable overlap between results derived from controls and from diseased patients, a finding entirely opposite to that made by Hinerman. The disparity between the two sets of figures made it necessary that we should verify our findings with an alternative technique, and this was accompanied by applying Ogilvie's method slightly modified in that it

TABLE 3.—*Estimation of Islet Diameter and Number with Eyepiece Micrometer* [5]

	No. Cases	No. Islets and Part Islets/Sq. Cm.	Mean Largest Diameter of 35 Consecutive Islets *
Normal †	10	42.4-99.2 (Mean 67.2)	128.8-156.5 (Mean 142.4)
Controls ‡	7	89.3-337.0 (Mean 168.9)	72.9-180.2 (Mean 111.5)
Addison's disease †	18	184.0-642.0 (Mean 327.0)	174.2-260.0 (Mean 201.5)
Addison's disease	16	73.8-431.0 (Mean 194.1)	80.3-159.8 (Mean 112.6)

* In microns.
† Hinerman.[5]
‡ This series.

TABLE 4.—*Estimation of Islet Number and Percentage Islet Area in Transverse Sections of Head, Body, and Tail of Pancreas* [10]

	No. Cases	No. Islets and Part Islets/Sq. Cm.	Percentage Islet Area
Normal, lean *	19	72.0-291.0 Mean 187.5	0.80-3.84 Mean 2.05
Normal, fat *	19	60.2-270.0 Mean 164.8	0.74-5.71 Mean 3.19
Controls †	3	169.0-275.0 Mean 204.6	1.56-3.5 Mean 2.38
Addison's disease †	8	160.5-371.0 Mean 229.0	1.296-3.69 Mean 2.47

* Ogilvie.
† This series.

involved planimetry. It will be seen from Table 4 that our figures and Ogilvie's were very similar, thus making any gross variation in islet tissue unlikely. The same inference may be drawn from a comparison of the islet "weights" deduced by this method.

Thus, in our eight cases of Addison's disease the islet organ "weighed" between 0.272 and 3.5 gm. (mean 1.187), that is, between 0.012 and 0.065 gm. per kilogram of body weight (mean 0.024). These figures are similar to those derived from three cases dying from other than endocrine diseases (1.01, 1.25, and 2.35 gm., i. e., respectively 0.021, 0.031, and 0.046 gm. per kilogram of body weight), and conform well with 44 similar cases aged between 15 and 57 years analyzed by Ogilvie.[10] He estimated that the islet organs weighed between 0.310 to 2.32 gm. (mean 1.044), i. e., between 0.01 and 0.045 gm. per kilogram of body weight (mean 0.022).

Other Pathological Findings.—Few other histological changes have been noted in the pancreas in Addison's disease, save when this condition has complicated diabetes mellitus.§ A slight lymphocytic infiltration was present in our Cases 3 and 4, but such changes were exceptional. As for the cytology of the islets, the hydropic change noted by Kraus [17] was not confirmed, nor were any counts performed which could verify the slight diminution in the number of B cells claimed by Hinerman. However, in two cases B granules were not demonstrated; in Case 1 for no obvious reason, in view of the otherwise normal cytoplasm of the islet cells, and in Case 3, presumably because the cytoplasm of all the islet cells was shrunken and the pancreas greatly reduced in weight (15.7 gm.). This patient has already been reported [13] as a case of diabetes mellitus complicating Addison's disease. There was, however, a marked hypoglycemia (0.050 gm. %) on admission three months before death, and hyperglycemia developed after repeated intravenous injections of adrenocortical extract. In view of the diabetogenic properties of certain of these extracts, the possibility must be entertained that hyperglycemia is sometimes therapeutic in origin. If, however, this were so, one would expect diabetes mellitus to supervene in Addison's disease rather oftener than is the case, for very few examples of this sequence have been reported.‖

Islet Adenomata.—The presence of islet adenomata is of interest, both because of the rarity of such tumors and because of their frequent association with hypoglycemia. There was, however, no means of determining in retrospect their role in our two cases of Addison's disease. Thus, the absence of any definite symptoms referable to hypoglycemia was not unexpected in a disease characterized by other and more obvious symptoms. Pathological evidence was equally equivocal. For, although the small size of our two tumors was somewhat against their causing severe attacks of hypoglycemia, it is clear that small tumors can do so.[24] It is equally difficult to interpret the cytological evidence, namely the absence of cells containing B granules from the islet adenomata and the presence of such cells in the normal islet tissue. For, in the case of tumors which are known to be secreting insulin, more often than not no B granulation can be demonstrated.[25] This is certainly our experience on postmortem and surgical material. As for the state of the adjacent islets, our attention was drawn to these by Gomori.[25] He observed B cell degranulation and hydropic change in islets in glands containing functioning islet tumors. Nerenberg [24] has recently greatly stressed this degranulation, attributing it to an excess of insulin, but he admits that such degranulation is inconstant, perhaps because it takes time to develop. This explanation is not wholly convincing, for in two cases with functioning islet tumors of long duration we have found abundant islet cells with normal B granules. A second feature of such tumors is hyalinization of the connective tissue as seen in Case 8. The importance of this feature as a means of recognizing the active tumor has recently been stressed by Shields Warren,[26] quoting a personal communication from Meissner, but Nerenberg's inability to confirm the constancy of this hyalinization seems to invalidate its use as a criterion. In short, the cytological changes seen in these two tumors seem consistent alike with activity and inactivity.

§ References 18, 19, and 20.

‖ References 21, 22, and 23.

However, whatever the funtional state of these tumors, their rarity is undeniable. Indeed, even if, as Pease [27] suggests, they are to be found in as many as 8 out of 500 necropsies (1.6%) their occurrence in 2 of 17 cases of Addison's disease (10.9%) can scarcely be attributed to chance. Assuming therefore that they were not fortuitous in our two cases, an explanation for their presence must be found. This is a matter for speculation. We are only familiar with one condition, namely diabetes mellitus, in which islet adenomata have been encountered with any frequency as an incidental finding.¶ For this reason, and because in Addison's disease there is a similar, if far less marked, tendency to pancreatic atrophy, we suggest that in both conditions islet adenomata may represent a focal attempt at compensatory islet hyperfunction.

CONCLUSION

Whatever the interpretation of these two islet adenomata, it must be stressed that they were the only changes in our series in the least compatible with the gross islet hyperplasia described by Hinerman. Indeed, even accepting the validity of his technique, we cannot agree that his findings establish any absolute increase in the amount of islet tissue. Consequently, we suggest that theories which involve islet hyperplasia and insulin oversecretion in Addison's disease are at least very highly speculative. At present the tendency in this condition towards hypoglycemia should be regarded as extrapancreatic in origin.

SUMMARY

The pancreas has been examined in 17 cases of Addison's disease.

Seven of the 15 weighed glands were of somewhat reduced weight.

Deductions based on the weight of the pancreas indicated in eight cases no gross abnormality in the amount of islet tissue.

Hinerman's contention that there is a gross hyperplasia of islet tissue in Addison's disease was not confirmed, even by the use of his technique.

Small islet adenomata were found in two cases. There was no clinical or cytological evidence to suggest whether these were functioning or not.

Prof. Dorothy Russell helped in the preparation of this paper, and Mr. Victor Trenwith provided technical assistance.

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EXTRAMAMMARY PAGET'S DISEASE OF THE VULVA

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DETROIT

EXTRAMAMMARY Paget's disease is rarely reported. When it is reported, usually the case report appears in the literature quite soon after the entity is noted by the pathologist or gynecologist. A recently reviewed case of this unusual entity seems worth presentation because of its many interesting features. Among these was the failure to demonstrate any close neoplastic involvement of the underlying tissue.

Paget's disease of the vulva is rare, and only casual mention, if any, is given in the leading textbooks of gynecologic and obstetric pathology. It is apparent now that it presents a distinct clinical and pathological picture. The classic work of Sir James Paget [1] in 1874 presented the possibility that the dermatological picture of the nipple area might be seen in other sites. It was for Darier and Wickham to record the microscopic picture by which Paget's disease can be definitely recognized. In 1937 when Weiner [2] reviewed the literature of extramammary Paget's disease he found 15 cases reported in sufficient detail to be classified as Paget's disease. This was from a total of 58 reported cases (including the one additional case he listed). Of these 15 cases, 7 occurred in areas other than the vulva and 8 were present in that location. Since 1937, seven additional cases have been reported. Traub [3] (1943); Casper [4] (1948);* DePrisco and Convit [5] (1948); Ahumada [6] (1950); Huber, Gardiner, and Michael [7] (1951), and Dockerty and Pratt [8] (1952) have all reported a case of Paget's disease of the vulva, except for the last two papers, which reported three and two cases respectively. This gives an estimated total in the literature of 16 reported cases of Paget's disease of the vulva.

REPORT OF A CASE

Mrs. S. E. (No. 264418), aged 42 years, was first seen at the Henry Ford Hospital in October, 1942. At that time she complained of burning and itching around the vulvar region for one year. This had become intensified in the past six months. On Oct. 12, 1942, a vulvectomy was performed. The gross pathological specimen was described as consisting of the mons pubis, labia majora, labia minora, and clitoris. It was noted that the skin of the labia minora and majora on the right had a mottled appearance (alternate red and white areas). The entire region appeared atrophic, and there was a slightly depressed margin at the junction of the surrounding skin.

From the Department of Laboratories, Henry Ford Hospital, Dr. Bowman Associate Pathologist; Dr. Hartman Pathologist in Chief.

* Both Traub and Casper presented the same case.

The microscopic sections taken from the various parts of the gross specimen demonstrated an intact epithelial surface and in focal areas marked hyperkeratosis. There were some areas of atrophy and elongation of the rete cones. The latter were filled with multiple masses of large undifferentiated cells sharply contrasting with the surrounding squamous epithelium. Extension of these cells beyond the epithelium was not observed. These cells were typical of so-called

Fig. 1.—Paget's disease of vulva. Note the nests of Paget's cells in the epidermis. There is associated hyperkeratosis and parakeratosis which is frequently found. × 60.

Fig. 2.—Paget's disease of vulva. Note the large vesicular nuclei. Intercellular bridges are absent. Pleomorphism is apparent. × 258.

Paget's cells. There was a marked round cell infiltration in the subjacent portions of the lamina propria. Specimens taken from the peripheral margins demonstrated no infiltration in the underlying surface.

This patient was again admitted in November, 1942, and at that time a bilateral dissection of the inguinal and femoral lymph nodes was performed. The pathology report of these resected

inguinal nodes failed to reveal evidence of neoplastic metastasis, and a diagnosis of chronic lymphadenitis was made.

The patient was followed at six-month intervals and had no recurrence of her malignant process in the vulva. In June, 1949, she was again admitted to Henry Ford Hospital. She was found to have a mass in the left lower hemisphere of the left breast, which had been present for approximately one year but in the past six weeks had become tender. There was no discharge from the nipple. The gross specimen measured 3 cm. in its greatest diameter when it was removed. A left radical mastectomy was performed.

Microscopic examination of the main tumor mass in the breast showed fairly small cuboidal or polygonal cells which tended to fill the small ducts with solid tumor masses. In many areas these had infiltrated beyond the confines of the duct and were infiltrating, extending to the fibrous and adipose tissue stroma, in the manner of a scirrhus carcinoma. Microscopic examination of sections from the axillary lymph nodes demonstrated metastases. Over a period of two months the patient received roentgen therapy to the anterior and posterior breast and supraclavicular areas as well as the axilla. The patient has been followed, and she remains well, demonstrating no recurrence in either the vulva or breast areas. It is now approximately 12 years since the diagnosis of Paget's disease of the vulva.†

COMMENT

The Paget cell was first described by Darier and Wickham; its source has been a question that has plagued pathologists and dermatopathologists ever since. Microscopically, Paget cells have an anaplastic appearance and are foreign to the epidermis. They are large, rounded, and generally well-defined. The cytoplasm is abundant and is either vacuolated or takes a light stain. The nuclei are hyperchromatic and may show mitotic figures. The Paget cells may appear in clumps and seem to lie in clear spaces. The questions have arisen time and again. Are these cells metastatic cells from a deep underlying adenocarcinoma? Is the presence of Paget's disease always an indication that there is an underlying adenocarcinoma? Is the presence of Pagetoid cells or Paget's cells a precancerous lesion, such as an intraepithelial carcinoma? Do Paget cells just happen in the epithelium? Practically all manners of theories and explanations have been presented. Darier [9] and Wickham [10] thought Paget's cells could be parasites, but the fallacy of their thinking was soon corrected. In recent years the idea has been again promulgated (Huber, Gardiner, and Michael) that Paget cells represent infiltrating malignant cells from an apocrine gland lesion underlying the epidermis. It was in 1917 that Sekiguchi [11] first attributed the lesion to an intraepidermal growth of carcinoma probably commencing in the sweat ducks. Pinkus and Gould [12] adequately differentiated Paget's disease from Bowen's disease and erythroplasia of Queyrat, with which many pathologists earlier confused it. Willis [13] states that extramammary and mammary Paget's disease are, like Bowen's disease, a form of primary epidermal carcinoma. Montgomery [14] believes he has seen transitions from basal and prickle cells to Paget's cells. On this basis Paget's disease would be an unusual type of dyskeratotic phenomenon. Broders and many others assume the multicentric origin of the cell and believe that the cell may arise from the basal cells of the epidermis and basal cells of the dermal appendages as well as the lactiferous ducts of the mammary gland.

Weiner,[2] in his excellent review of the literature up to 1937, felt that the Paget cell is definitely a cancer cell and that it occurs only in sites where there are sweat glands of the apocrine variety, but he does not offer evidence in his paper of transition from apocrine glands or changes in the apocrine cells in relation to Paget cells.

† Dr. Robert Parr, Visiting Surgeon to the Henry Ford Hospital, reviewed the case history.

He pointed out that the mammary glands are also modified sweat glands. Dockerty and Pratt [8] in their recent article point out even more clearly the need for an exhaustive search for a close underlying carcinoma when a Pagetization of skin occurs. They also demonstrated by histochemical technique that the Paget cells were true signet-ring cells and submitted the thesis that they migrated into the skin from deep-growing mucus-containing cells of glandular origin. They felt that this supported the concept that the intradermal changes of Paget's disease are metastatic.

Clinically the eczematoid lesion of extramammary Paget's disease is distinguished by well-defined borders and an appreciable degree of induration. The surface becomes congested and red, with weeping and oozing, but unlike true eczema the borders remain sharply defined. The process of induration and infiltration gradually occurs. In almost all instances of recorded cases of Paget's disease of the vulva, pruritus and burning was the primary complaint. The average life span is 67 years, with a range from 33 years to 84 years. The average duration of the pruritus and burning varied from 17 years to a few months. In approximately half of the cases a deep underlying adenocarcinoma was later discovered. In over half the cases a vulvectomy was performed after the diagnosis of Paget's disease, and in one-third of the cases roentgen therapy was administered to the urea. Of the cases reported in the literature, it is presumed that our patient is the only one which remains alive to date. (The possible exception is the case reported by DePrisco and Convit of a 32-year-old woman in 1948 whose lesion resembled leucoplakia.)

SUMMARY

This paper reports the 17th case of extramammary Paget's disease of the vulva. This case is unusual in the respects that (*a*) the patient is alive and well 14 years following the diagnosis of this lesion and (*b*) the fact that the patient later developed an adenocarcinoma of the mammary gland. It is our hope that with the addition of more cases to the literature a trend or pattern may be established so that the true nature of this peculiar eczematoid picture with its characteristic cells may be better understood. It is our contention that extramammary Paget's disease represents a primary malignant process of the quality of a "Grade I epidermoid carcinoma." Multiple sections were taken of the tissue removed from the original vulvectomy. However, in no section did we see an associated adenocarcinoma of an underlying apocrine gland. (Perhaps, if so-called true serial sections had been performed we would have been able to demonstrate such a lesion.) It is our feeling that the idea set forth by Huber, Gardiner, and Michael is probably the correct one—i. e., the Paget cell originates by extension from an underlying tumor of an apocrine gland. If this were not so, one would be hard-pressed to explain the failure in so many instances to find a deeper underlying adenocarcinoma. In all probability, with the biopsy excision or the vulvectomy, the offending slow-growing adenocarcinoma is removed but not discovered when sections are made of the lesion. The situation in the breast, we feel, is somewhat different because here the glandular structures are somewhat larger and more easily demonstrated. We do not agree with Montgomery or with Willis that Paget's disease is another form of, or is true, dyskeratosis of the epidermis.

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LIVING MESENTERIC TERMINAL ARTERIOLES BEFORE AND IMMEDIATELY AFTER EMBOLIZATION

Measurement and Comparison of Internal Diameters; A Contribution to the Biophysics of Disease

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INTRODUCTION

THE PURPOSE of this paper is to present a series of measurements in the CGS (centimeter, gram, second) system which can be of use in further studies of the biophysics of disease. The measurements should prove immediately useful in studies of the deformation and flow characteristics, or rheology, of unagglutinated and of pathologically agglutinated, or sludged, blood. Specifically, the purpose of the paper is to present measurements made in vivo of the internal diameters of the narrowest portions of the peripheral vascular bottlenecks, sometimes called the terminal arteriole precapillary sphincters, of the mesenteric vessels of frogs, hamsters, mice, rabbits, cats, and dogs under two separate conditions: first, during phases of healthy blood flow when the lumen of this narrowest portion is patent, and completely unagglutinated blood is flowing rapidly and easily through into the capillary bed; second, during periods when the same or similar bottlenecks are plugged by one or more masses of sludge.

Minute emboli, both sterile and infected, are of course well known to morphologic pathologists. However, the actual dimensions of masses which are just too large to pass through terminal arterioles of any species are not known.* Thus, one phase of the problems presented by sludged blood is to determine the maximal sizes which the narrowest parts of the bottlenecks can attain before or as each becomes plugged with one or more masses of sludge.

The logical necessity for making this study and the position of its results in a series of investigations can be indicated by listing and describing certain observations, as follows:

1. During health the circulating red cells of animals and man are not agglutinated but actually tend to repel one another slightly. See (Knisely, Stratman-Thomas, Eliot, and Bloch [1945] [98]; Knisely, Bloch, Eliot, and Warner [1950],[95] and Knisely [1951] [92]).

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An extension of thesis work presented in partial fulfilment of the requirements for a Master's Degree in Anatomy, Graduate School of the Medical College of South Carolina.

* For one important aspect of this problem, see Hicks and Warren, p. 409.

2. Intravascular agglutination of the circulating blood, or sludged blood, has been found to exist and be a part of the pathologic physiology of a number of the diseases and pathologic states of animals and of man (older literature, Fahraeus [1929] †; more recent literature, Knisely, Bloch, Eliot, and Warner [1947][94]; Knisely, Bloch, Eliot, and Warner [1950],[95] and Knisely [1951] [92]).

3. Geometrically, arterioles and terminal arterioles are segments of long, slowly tapering, truncated cones. This has been proved by Jeffords,‡ who carefully measured the internal diameters of mesenteric arterioles in living frogs and mice. Every vessel measured was found to be a cone, with the angle of taper varying from 7 minutes to 2 degrees 22 minutes in frogs and from 5 minutes to 2 degrees 17 minutes in mice.

The geometric shapes of terminal arterioles are of fundamental importance in dealing with the real problems of the rheology of normal and pathologic blood. Knisely, Bloch, Eliot, and Warner (1947) [94] were the first to understand the meaning of these geometric forms in pathologic circulatory physiology and the influence which the cone shape must have upon the dissipation of arterial pressure uselessly as agglutinated blood flows through the cones. They say:

> The shapes and dimensions of the vessels of the peripheral vascular beds constitute a perpetual bottleneck in the circulatory system. All the vessels which carry blood toward tissues are long, narrow, slowly tapering, truncated cones. When arterioles branch, each branch has a narrower lumen than that of the parent stem. The capillaries or sinusoids which carry blood through tissues are approximately cylinders, and the veins are slowly widening cones again. Arteriovenous anastomoses have been found in some tissues, but these have small total surface areas; if oxygen, glucose, and other blood-borne anabolites are to be distributed effectively to the cells of a tissue, the blood must flow through the capillaries or sinusoids of that tissue.

Knisely (1951) [92] further says:

> The resistance of sludged blood to its own passage through the bottlenecks of the circulatory system forcibly reduces the rates of blood flow through all open vessels of the body. The larger and the more rigid the masses of which the sludge is composed, the more the rates of flow are decreased. This forcibly reduces the rates at which the oxygen and glucose of the blood can be carried to the endothelium of the walls of all small vessels of the body.

4. The terminal arterioles and other portions of peripheral vascular bottlenecks have a limited degree of distensibility. The physical properties of the materials of which the walls of the various types of arteries are composed determine the distensibility of the arterial bottlenecks. Arterioles are made up of the three concentric zones which are common to all arterial vessels: an intima, composed of the endothelium and connective tissue; a media, composed for the most part of smooth muscle, and an adventitia, made up of loose connective tissue.

As one traces along a small artery and its branches toward the capillaries, the media, consisting of smooth muscle, becomes progressively thinner. Near the termination of the arteriole the endothelium is present and the media is no longer a continuous coat of muscle tissue but consists of smooth muscle bundles around the

† References 49 to 51.

‡ Jeffords, V., work in preparation.

arteriole. Strong (1938) [144] described and illustrated the architecture of this region precisely. The smooth muscle bundles form a series of short sleeves or rings of muscle tissue. At the junction of the terminal arterioles and the beginning of the capillaries there is a final ring of smooth muscle fibers around the tubular endothelial lining. This last muscle ring is sometimes called the precapillary sphincter.

The fundamental importance of the region of the precapillary sphincter is that at this point, or along a small region in this general neighborhood, the arteriole, and the peripheral vascular system itself, has its smallest anatomical internal diameter. Every molecule of every blood-borne anabolite which reaches the cells of each tissue of the body must come down through one or another of these extremely narrow terminal arteriole bottlenecks. Further, this terminal portion cannot dilate indefinitely, since the connective tissue fibers, which surround the muscle rings, have a limited distensibility.

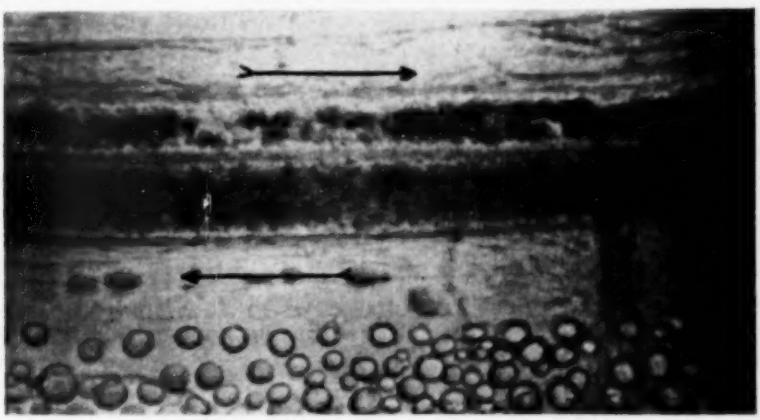

Fig. 1.—Sludged blood in a small mesenteric arteriole (upper vessel) of a young cat. A general estimate of the magnification of the picture can be obtained by noting the fat cells all along the lower portion of the picture. The sludge was initiated in this animal by crushing one of its hind legs. Photographed by Strobe-flash illumination; duration of exposure not more than 1/1,000 of a second. The picture shows that with high speed photomicrography presence or absence of masses of sludge in flowing arterial blood can be recorded and demonstrated.

With an understanding of these facts, it is now possible to discuss the significance of the cone-shaped arterioles. In at least some pathologic conditions the blood in the arterial system, including that passing down terminal arterioles, is agglutinated. Some observers have felt that in disease only the blood in small veins is agglutinated. Arterioles are ordinarily narrower than their corresponding venules; hence, linearly the blood in arterioles goes more rapidly than the blood in corresponding venules, often making direct microscopic observation of the masses in arterial blood very difficult. High speed microphotography, however, clearly demonstrates the presence of sludge in arterioles. Figure 1 shows such sludge produced in a cat by crushing the muscles of one leg (Knisely, Eliot, and Bloch [1945] [97]; Knisely, Bloch, Brooks, and Warner [1950] [93]; Laufman, Martin, and Tanturi

[1948],[101] and Heimbecker and Bigelow [1950] [73]). Note that the masses in the arteriole are much larger than the masses in the venule. In this particular case, the masses of sludge in the arteries were being broken up as they passed through terminal arterioles into the smaller masses which can be seen in the vein.

The masses of agglutinated blood cells in a sludge may be divided into two separate classes. The first are the basic masses, each of which by definition has sufficient internal cohesion so that it is not broken up into smaller masses as it passes down the long, progressively narrower terminal arterioles through the capillaries, venules, etc. The other type of mass consists of aggregates of basic masses held together with varying degrees of tightness. These larger aggregates have usually been called "charge aggregates" because frequently basic masses can be seen to migrate toward one another in vivo in stationary pathologic human blood (for method of observation see Knisely, Bloch, Eliot, and Warner [1950][96]). As far as our background knowledge of physics permits us to judge, such migrations of masses within and through otherwise stationary (not flowing) blood could occur only by mutual attraction of the masses.

By electron microscopy, Bloch and Powell (1952) [19] have found coating materials on red cells from some human beings with agglutinated blood. One problem today is to determine (for each pathologic condition and disease of each host species in which blood is agglutinated) whether the agglutinated blood cell masses are held together by sticky substances, by electric charges, or by other materials or forces (for discussion, see Knisely [1951] [92]).

For the following discussion, it is important to separate sharply the physical properties of basic masses from those of charge aggregates.

As charge aggregates are carried into progressively narrower arteries and forced into vessels too narrow to accommodate them, the charge aggregates frequently are first deformed and then broken up into basic masses.

When semi-rigid basic masses of agglutinated blood cells are carried into and forced down progressively tapering arterioles, the basic masses are compressed from shapes which may approach that of a cube or irregular sphere toward shapes which approach cones or spindles. Many basic masses are, of course, compressed sufficiently to pass through terminal arterioles and on through capillaries and venules.

As fairly rigid basic masses are carried into and forced down into terminal arterioles, they frequently or usually bulge the vessel somewhat and simultaneously are compressed to the spindle shape by the vessel.

If we consider a series of basic masses, two factors stand out: One is the actual size of the mass when it is not compressed, that is, when it is floating freely within a blood vessel which can easily accommodate it; the other variable is the rigidity of the basic mass. A handful of absorbent cotton is fairly soft. A tennis ball is more rigid than cotton, and a golf ball is more rigid than a tennis ball. Thus, for this purpose "rigidity" may be defined as resistance to deformation and compression. These two properties of the basic mass, its diameter and its degree of internal rigidity, operating simultaneously, determine the resistance which the mass pre-

sents to being pushed down each progressively narrower portion of the terminal arteriole.

In some cases, at least in some species, the masses of sludge formed after crushing injuries are large and somewhat soft. In contrast, the masses in the same species formed after severe burns are much smaller but much more rigid.§ Most masses of each type pass through terminal arterioles. However, a slight increase either in the size or in the rigidity of such masses causes them immediately to act as direct emboli. Thus, the two factors, the diameter and the rigidity, must simultaneously be considered when dealing with the "flow characteristics" of a sludge.

As a basic mass which is sufficiently large and rigid to embolize a terminal arteriole is carried down and forced into the cone-shaped bottleneck, the mass forcibly bulges the vessel before and, as the mass impacts, plugs and embolizes it. Hence, the firmly embolized vessel has a somewhat greater internal diameter immediately after embolization and impaction than it had before.

The phenomena described above lead to the need for asking the following questions:

1. What, in each of a series of species, including man, are the internal diameters of the narrowest portions of the terminal arterioles in the several tissues and organs of the living body when the animal is therapeutically untreated and the vascular system is conducting unagglutinated blood?

2. What are the actual internal diameters of the narrowest portions of the terminal arterioles when these are maximally relaxed and maximally forcibly distended?

3. What are the diameters of the largest masses which will just pass through these bottlenecks when the animal is therapeutically untreated (no vasodilator drugs, such as Dibenamine, for instance)?

4. What are the diameters of the largest agglutinated blood cell masses which will just pass through the arteriole bottlenecks when the masses are forcibly compressed and at the same time the bottlenecks are forcibly dilated by the passing masses?

Obviously, this investigation cannot provide answers to all these questions, but it is a first direct step toward making physical measurements of the narrowest portions of the peripheral vascular bottlenecks before and after embolization.

The original purpose of this investigation was to measure the internal diameters of the bottlenecks while unagglutinated blood was flowing through, and then to measure the diameters of the largest masses which would just pass through and the smallest masses which just plug. It is necessary to report the findings on the diameters of the bottleneck before and after plugging rather than the diameter of the mass which causes the plugging because, as noted, the masses which come through ordinarily are a little soft and change diameter during and after they embolize the arteriole tip. Thus far, no method has been designed by which the degree of rigidity of a mass can be measured in physical terms in vivo. Funda-

§ Unpublished observations from our laboratory.

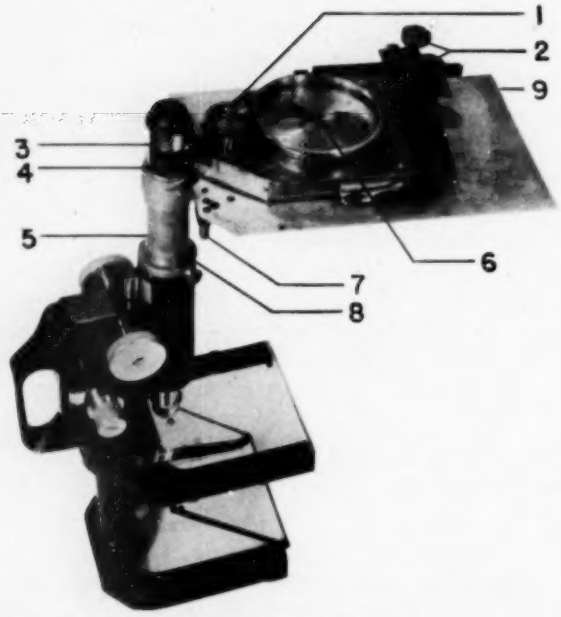

Fig. 2.—The micrometer and microscope viewed obliquely.

1, micrometer pointer adjustment knob. Turning this knob actuates a cam which forces the jaws of the micrometer to approach each other. The distance between the jaws is registered on the dial of the instrument.

2, mechanical stage adjustment knobs which move the whole micrometer on the large horizontal plate. These two knobs move the micrometer precisely in two planes.

3, the "cut-out" eyepiece. The cut-away section of this ocular permits the jaws of the micrometer to be maneuvered within the microscope ocular. The pointers mounted on the micrometer jaws are continually at the level at which a hair is ordinarily mounted in a microscope ocular when the hair is used as a pointer.

4, the micrometer jaws within the cut-away ocular.

5, the lengthened draw tube.

6, the scale of the micrometer. There is a train of gears between the jaws of the micrometer and the micrometer scale. This train of gears is spring-loaded in such a fashion that only one side of each tooth of each gear ever comes in contact with the teeth of other gears. This completely prevents mechanical "back lash" in the instrument.

7, micrometer holder rod. This vertical pin supports the aluminum plate (Item *9*, below) and thereby permits the jaws of the micrometer to be rotated within the ocular. Thus, this pin, together with the two knobs of the upper mechanical stage (Item *2*, above), gives complete control of the position of the jaws of the micrometer within a horizontal plane in the microscope ocular.

8, draw tube-supporting sleeve.

9, micrometer holder, really an upper flat stage.

The photograph shows the whole apparatus mounted on a microscope which has a regular specimen stage. Often, when the apparatus is used, it is mounted on a horizontal swing arm which has joints to permit the microscope to be pointed in any direction. This gives much more flexible control of the position of the whole apparatus and permits measurement of vessels running in any direction within the tissue (Fig. 4).

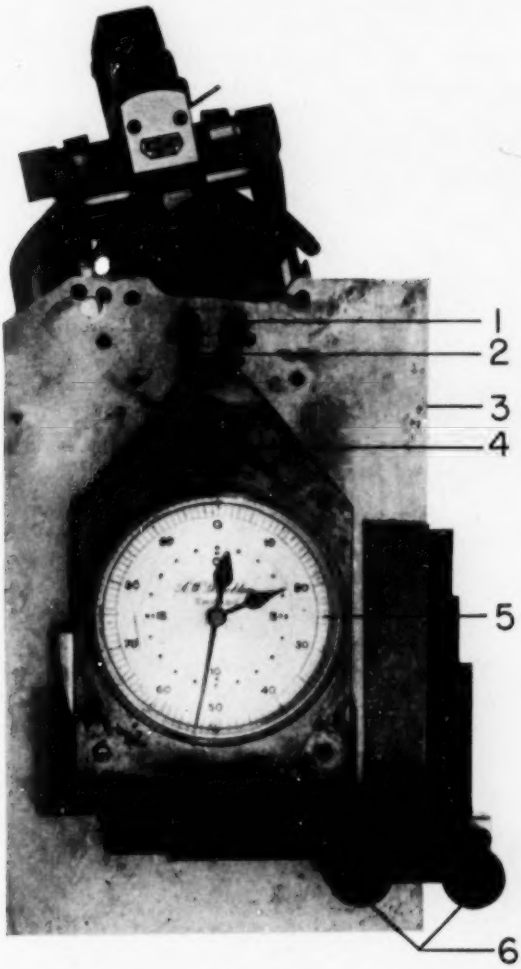

Fig. 3.—The micrometer as attached to the microscope, viewed from above.

The whole micrometer has been withdrawn from the ocular of the microscope in order to show the fixed and movable pointers which operate within the microscope ocular.

1, the movable pointer.

2, the fixed pointer.

3, the aluminum plate, which acts as an upper stage or as a micrometer holder.

4, the micrometer pointer adjustment knob.

5, the micrometer scale. The small hand on this dial registers the number of turns made by the large hand.

6, the mechanical stage adjustment knobs, which give precise movement of the whole micrometer in two planes.

As noted in Figure 2, the microscope ocular has been cut away, giving space to insert the fixed and movable pointers.

mental concepts of rigidity and deformability of solid bodies can be found in Houwink (1937) [82] and Sommerfeld (1950).[142]

As has been noted, minute emboli, both sterile and infected, are well known to morphologic pathologists. However, the actual dimensions of masses which are just too large to pass through terminal arterioles of any species are not known.

Thus, one phase of the problems presented by sludged blood is to determine the maximal sizes which the narrowest parts of the bottlenecks can attain before or as each becomes plugged with one or more masses of sludge.

Fig. 4.—Microscope with micrometer attached, mounted on a swing arm which permits microscope to be pointed in any direction. Note water-immersion lens projecting from bottom of microscope tube.

Heavy lead-filled tripod base and short horizontal arm minimize vibration of the microscope.

MATERIALS AND METHODS

A special ocular micrometer has been devised, the accuracy of which is limited only by the resolving power of the lenses in use on the microscope. All parts of the apparatus are attached to the microscope, and the micrometer itself measures the microscope image in the ocular at the level at which a hair is ordinarily inserted as a pointer. Thus, one most significant feature of the whole experimental arrangement is that parts of living tissues to be measured are not touched by any device whatsoever (Figs. 2 to 5).

The whole ocular micrometer rests on a flat aluminum plate, which is attached to the upper part of the barrel of the microscope (Fig. 2). An ordinary mechanical

stage mounted on this flat plate permits movement and precise control of the positions of the jaws of the micrometer in the microscope ocular. The aluminum plate can be rotated around a vertical pin (*7*, Fig. 2). Thus, the micrometer jaws can be superimposed on the ocular image of a blood vessel regardless of the angle at which the vessel crosses the microscope field.

The ocular micrometer has a fixed and a movable pointer, which are viewed in the ocular lens. The two pointers can be adjusted to converge on the image of the object to be measured by use of the various adjustment knobs which are incorporated into the system. The instrument was designed specifically to permit all

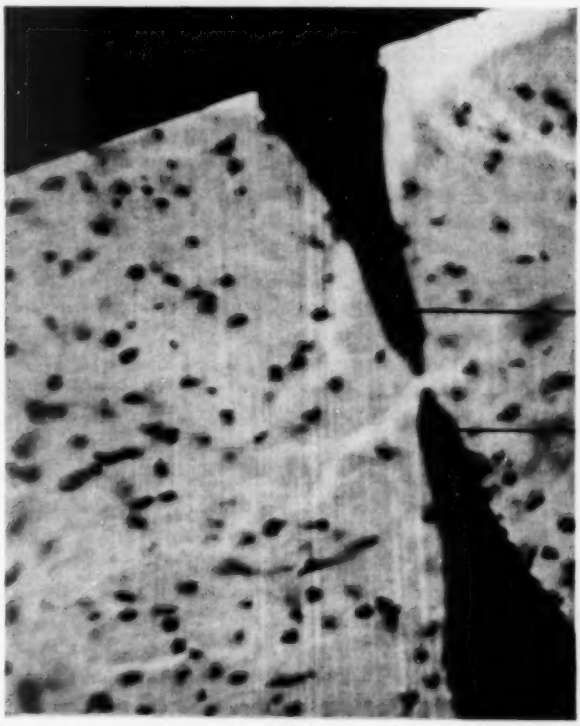

Fig. 5.—Appearance of the micrometer pointers in the ocular lens of the microscope as an object is being measured. The specimen photographed to make this picture was a histological section. *1* and *2*, the fixed and movable pointers, respectively.

mechanical adjustments to be made rapidly in order to make frequent measurements of histological structures which are changing dimensions rapidly (Fig. 5).

The movable pointer travels through the arc of a circle; the desired measurement is the chord of this arc. However, owing to the large ratio between the radius of the arc and the length of the chord used for these measurements, the difference between the arc of the circle used and the chord of this arc is less than the accuracy of the last digit quoted in the data; thus this inconsistency is absorbed.

The ocular micrometer was calibrated many times during the course of the work in order to be certain that the calibration values do not change between

sets of measurements. To calibrate it, 10 measurements were made of a known distance which consisted of the spaces on a standard calibrated American Optical Company glass slide. The 10 ocular micrometer figures were then averaged, and from this average the equivalent of the empirical scale of the ocular micrometer was determined in micra. The following is a sample list of 10 rapidly made measurements of a 0.01 mm. (10 μ) distance.

	Micrometer Units
1	208.5
2	212.0
3	211.0
4	209.0
5	208.0
6	212.0
7	206.5
8	208.0
9	205.0
10	208.5
	2,088.5 = Total

Average = 208.85 units = 10 μ

Thus, one ocular micrometer scale unit is equal to 10 μ, divided by 208.85, or 0.0478. Or, to the nearest significant figure, one micrometer scale unit equals 0.05 μ.

The largest of these measurements was 212 units and the smallest was 205 units, giving a difference of 7 units. If each unit is equal to 0.05 μ, then the difference between the largest and smallest measurements was 0.35 μ. The resolving power of the lenses used was less accurate than 0.35 μ. Thus the accuracy of the measurements is determined and limited by the resolving power of the lenses rather than by the mechanical properties of the micrometer.

The observations on frogs were made using a Leitz objective lens with a magnifying power of 20 and a 6-magnification ocular lens with a lengthened draw tube, giving a total magnification of approximtely 200 diameters. The observations on mammals were made using a Leitz water immersion objective with a magnifying power of 50 and a 6-magnification ocular with a lengthened draw tube, giving a total magnification of approximately 450 diameters.

Considering the lenses used for making the observations and measurements recorded, it was believed best to report the measurements as accurate to the nearest 0.5 μ.

As previously noted, frogs, hamsters, mice, rats, rabbits, cats, and dogs were used for this work. All the animals were healthy. The frogs did not have red leg disease; all the mammals had been kept and observed in our animal colony for some time before use.

Frogs were anesthetized with a solution of 25% urethan injected into the dorsal lymph sac. Mammals were anesthetized with pentobarbital (Nembutal) sodium 26.4 mg. per kilogram of body weight, given intraperitoneally.

All animals were anesthetized deeply enough so that respiration was slowed and the winking reflex lost. The animals were opened by a relatively bloodless and sludgeless laparotomy (Knisely, Eliot, and Bloch [1945] [97]). The mesenteries were exteriorized and placed on a holder which was designed to give maximum support and at the same time to allow transillumination of the tissues. The vessels of the mesenteries were observed in all species. In frogs the vessels of the bladder were studied in addition.

The studies were carried out with use of a quartz rod transilluminator, a device which has been described in a number of detailed papers.‖

‖ References to the fused quartz rod method are listed in Section II, page 18, of "An Annotated Bibliography on Sludged Blood" by Knisely (1951),[92] and by Loeser (1953).[102]

Under the experimental conditions imposed on these animals, spontaneous vasomotion, such as has been described by some observers, seldom occurred in the vessels we studied and measured.

In carrying out an experiment, a suitable microscope field was found and an arteriole with a patent lumen selected. The micrometer was adjusted to the inner surface of the inner wall of the arteriole (which is sharply refractile) and moved along the arteriole until the smallest diameter of the bottleneck was found. The smallest diameter is usually at the region of the precapillary sphincter. First, a series of measurements was taken from several patent vessels; then the animal was lightly traumatized by crushing an extremity (Knisely, Eliot, and Bloch [1945] [27]), or by the application of mechanical trauma near the artery leading to the vessels being measured, or by the application of heat to one of the animal's extremities (Finney [1950] [52] and Brooks, Dragstedt, Warner, and Knisely [1950] [28]). The mechanical or thermal trauma used was minimal in order to produce a mild sludge only. The masses of sludge were made to increase in size and number by applying additional trauma, and the trauma was continued until the masses of agglutinated blood cells became large enough to plug the bottleneck. The larger masses of the sludge formed by these methods always embolized, that is, plugged, the region of the precapillary sphincter.

The internal diameter of the plugged sphincter was then measured. Measurements from the same vessel when patent and later when plugged were made whenever possible, as the tables of data show; also, additional measurements were taken on different terminal arterioles, some before and others after embolization.

It is important to note that the frogs used were anesthetized with urethan and the mammals with pentobarbital sodium, as each of these substances has mild degrees of vasodilator properties. In the selection of the precapillary bottleneck in the patent state, an arteriole was studied during a time when blood was flowing smoothly through its lumen and there was little, if any, deformation of the red blood cells passing through. The precapillary bottlenecks were selected in this state because in our experience this approaches the largest diameter which these vessels attain in healthy animals when they are not subjected to specific vasodilator substances. (It is also most important to note that unagglutinated blood flows through such precapillary bottlenecks when these are contracted somewhat narrower than the recorded figures. Krogh [1929] [99] noted the folding of red cells passing through arterioles. Fulton and Lutz [1941] [56] have recorded this in motion pictures.)

As noted, the "plugged measurements" were made from the internal diameters of the plugged precapillary bottlenecks. Because the consistency or internal rigidity of the masses which make up the sludged blood cannot yet be measured in vivo, it was necessary to measure the diameter of the plugged precapillary bottleneck after this vessel was embolized.

It is important to note that these diameters may have been affected slightly by the anesthesia. The vasodilator effects of the anesthetic may perhaps also have affected the degree of relaxation of the precapillary bottlenecks. But since our major interest was in determining the maximum degree to which these vessels dilate when embolized, the anesthetic in this case assisted us in putting each vessel into the condition in which we wished to measure it.

RESULTS

The results of the measurements are presented in Tables 1 to 14.

TABLE 1.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Ten Frogs*: Measurements Made During Patent and Plugged Condition upon Same Vessel*

Vessel No.	Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference, μ
1	16.9‡	20.0	3.1§
2	16.2	19.5	3.3
3	16.4	19.6	3.2
4	16.7	20.4	3.7
5	15.3§	19.5	4.2‡
6	16.6	20.2	3.6
7	16.2	20.4	4.2
8	16.0	20.0	4.0
9	16.1	19.6	3.5
10	15.6	19.2§	3.6
11	15.9	19.5	3.6
12	16.4	20.6‡	4.2
13	16.6	20.6	4.0
14	16.3	20.5	4.2
15	16.1	19.7	3.6
Averages	16.2	20.0	3.8

* Anesthetized with urethan.

† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 16.9 μ, is smaller by 2.3 μ than the smallest diameter, 19.2 μ, recorded for a plugged vessel. The two series of measurements do not overlap.

‡ Largest.

§ Smallest.

TABLE 2.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Ten Frogs*: Measurements Made During Patent and Plugged Condition upon Different Vessels But All of Same Anatomical Class*

Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference in Average of Patent and Plugged Vessels, μ
16.3	19.8	
16.9‡	19.6	
15.3	20.0	
15.3	21.4	
16.6	21.4	
16.8	21.1	
16.2	21.2	
16.8	21.6‡	
14.9§	19.1§	
15.0	21.0	
15.6	20.0	
16.0	21.0	
15.0	19.9	
16.0	20.3	
15.0	20.0	
15.8	19.6	
15.8	19.8	
16.1	19.6	
15.6	20.0	
16.0		
15.8		
16.1	20.2	4.1 (Averages from Tables 1 and 2)

* Anesthetized with urethan.

† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 16.9 μ, is smaller by 2.3 μ than the smallest diameter, 19.1 μ, recorded for a plugged vessel. The two series of measurements do not overlap.

‡ Largest.

§ Smallest.

TABLE 3.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Ten Hamsters*: Measurements Made During Patent and Plugged Condition One Each on Same Vessel*

Vessel No.	Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference, μ
1	6.2	9.4	3.2
2	6.4‡	9.1	2.7
3	6.4	9.2	2.8
4	6.0§	9.1	3.1
5	6.3	9.3	3.0
6	6.1	9.2	3.1
7	6.4	9.4	3.0
8	6.2	9.2	3.0
9	6.4	9.0	2.6
10	6.0	9.5‡	3.5‡
11	6.3	8.8§	2.5§
Averages	6.2	9.1	2.9

* Anesthetized with phenobarbital (Nembutal).

† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 6.4 μ, is smaller by 2.4 μ than the smallest diameter, 8.8 μ, recorded for a plugged vessel. The two series of measurements do not overlap.

‡ Largest.

§ Smallest.

TABLE 4.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Ten Hamsters*: Measurements Made During Patent and Plugged Condition upon Different Vessels But All of Same Anatomical Class*

Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference in Average of Patent and Plugged Vessels, μ
6.4	9.2	
6.2	9.0	
6.0‡	9.2	
6.3	8.6‡	
6.2	9.1	
6.2	9.2	
6.2	9.4	
6.2	9.0	
6.4	9.1	
6.2	9.0	
6.3	9.1	
6.2	9.2	
6.5	9.2	
6.2	9.4	
6.0§	9.0	
6.4	8.9	
6.2	9.1	
6.3	9.3	
6.2	9.1	
6.1	9.6	
6.4	9.4	
6.1	9.4	
6.1	9.7§	
6.4	9.2	
6.0	9.6	
6.5	9.3	
6.2		
6.1		
6.5		
6.4		
6.4		
6.4		
6.3	9.3	3.0 (Averages from Tables 3 and 4)

* Anesthetized with phenobarbital.

† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 6.6 μ, is smaller by 2.0 μ than the smallest diameter, 8.6 μ, recorded for a plugged vessel. The two series of measurements do not overlap.

‡ Smallest.

§ Largest.

TABLE 5.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Seven Mice*: Measurements Made During Patent and Plugged Condition upon the Same Blood Vessels*

Vessel No.	Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference, μ
1	6.2	9.6‡	3.4‡
2	5.6	8.7	3.1
3	5.4	8.4	3.0
4	5.2§	8.1§	2.9
5	5.7	8.6	2.9
6	6.6‡	9.3	2.7§
Averages	5.7	8.7	3.0

* Anesthetized with phenobarbital.
† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 6.6 μ, is smaller by 1.5 μ than the smallest diameter, 8.1 μ, recorded for a plugged vessel. The two series of measurements do not overlap.
‡ Largest.
§ Smallest.

TABLE 6.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Seven Mice*: Measurements Made During Patent and Plugged Condition upon Different Vessels But All of Same Anatomical Class*

Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference in Average of Patent and Plugged Vessels, μ
6.1	9.1	
6.4	9.0	
6.2	9.7‡	
6.0	8.7§	
6.4	9.2	
6.8‡	9.1	
6.2	9.0	
5.2§	8.7	
6.2	9.1	
5.3		
6.0		
5.5		
6.4		
6.1		
6.4		
5.2		
5.9	8.9	3.0 (Averages from Tables 6 and 7)

* Anesthetized with phenobarbital.
† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 6.8 μ, is smaller by 1.9 μ than the smallest diameter, 8.7 μ, recorded for a plugged vessel. The two series of measurements do not overlap.
‡ Largest.
§ Smallest.

TABLE 7.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Five Rats*: Measurements Made During Patent and Plugged Condition upon Same Vessel*

Vessel No.	Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference, μ
1	5.6‡	8.0	2.4
2	6.0	8.6§	2.6§
3	6.2§	8.2	2.0
4	5.9	8.0	2.1
5	5.9	8.2	2.3
6	5.8	8.4	2.6
7	6.2	7.8‡	1.7‡
8	6.0	8.4	2.4
Averages	5.95	8.20	2.25

* Anesthetized with phenobarbital.
† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 6.2 μ, is smaller by 1.6 μ than the smallest diameter, 7.8 μ, recorded for a plugged vessel. The two series of measurements do not overlap.
‡ Smallest.
§ Largest.

TABLE 8.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Five Rats*: Measurements Made During Patent and Plugged Condition upon Different Vessels*

Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference in Average of Patent and Plugged Vessels, μ
5.9	8.3	
6.1	8.8‡	
6.2	7.9	
6.0	8.7	
6.2	8.2	
5.2§	8.0	
6.0	8.2	
5.8	8.6	
5.8	7.9	
6.0	8.0	
6.3	8.2	
6.3	8.4	
5.7	7.7§	
6.0	8.0	
6.2	7.9	
6.2	8.5	
5.8	8.4	
5.7	8.2	
5.8	8.3	
6.1	7.7	
6.1	7.9	
6.0	8.1	
6.2	7.8	
6.3	7.7	
6.4‡	7.9	
5.7	7.8	
5.8	8.4	
6.2	8.6	
5.9	7.7	
6.4	8.5	
6.01	8.16	2.15 (Averages from Tables 7 and 8)

* Anesthetized with phenobarbital.

† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 6.4 μ, is smaller by 1.3 μ than the smallest diameter, 7.7 μ, recorded for a plugged vessel. The two series of measurements do not overlap.

‡ Largest.

§ Smallest.

TABLE 9.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Five Rabbits*: Measurements Made During Patent and Plugged Condition upon Same Vessel*

Vessel No.	Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference, μ
1	5.8‡	8.1	2.3§
2	5.2§	7.7§	2.5
3	5.6	8.4‡	2.8‡
Averages	5.52	8.07	2.55

* Anesthetized with phenobarbital.

† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 5.8 μ, is smaller by 1.9 μ than the smallest diameter, 7.7 μ, recorded for a plugged vessel. The two series of measurements do not overlap.

There are only three sets of measurements in this series because of difficulty in holding the tissue still. Note that the maximum, minimum, and averages fall within the range of similar figures recorded in Table 10.

‡ Largest.

§ Smallest.

TABLE 10.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Five Rabbits*: Measurements Made During Patent and Plugged Condition upon Different Vessels*

Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference in Average of Patent and Plugged Vessels, μ
5.4	8.0	
5.6	7.6	
5.4	8.2‡	
5.6	8.1	
5.8	7.8	
5.2	7.8	
5.5	8.0	
5.4	8.0	
5.6	8.0	
5.2	8.2	
5.8	7.7	
5.4	8.2	
5.6	8.2	
5.4	7.9	
5.6	8.0	
5.2	8.0	
5.4	8.1	
5.9‡	7.2§	
5.8	8.0	
5.3	8.2	
5.4	8.0	
5.6	7.8	
5.8	8.1	
5.2	7.8	
5.6	7.9	
5.5	7.7	
5.4	7.8	
5.7	8.2	
5.3	8.0	
5.4	8.2	
5.8	8.0	
5.5	8.2	
5.6		
5.4		
5.9		
5.0§		
5.1		
5.6		
5.4		
5.6		
5.50	8.06	2.56 (Averages from Tables 9 and 10)

* Anesthetized with phenobarbital.

† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 5.9 μ, is smaller by 1.3 μ than the smallest diameter, 7.2 μ, recorded for a plugged vessel. The two series of measurements do not overlap.

‡ Largest.

§ Smallest.

TABLE 11.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Five Cats*: Measurements Made During Patent and Plugged Condition upon Same Vessel*

Vessel No.	Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference, μ
1	8.0	11.2	3.2
2	8.4	11.6	3.2
3	7.9	11.8‡	3.9
4	7.7	11.4	3.7
5	8.0	10.9§	2.9
6	7.8	11.7	3.9
7	7.8	11.6	3.8
8	8.2	11.2	3.0
9	8.6‡	11.8	3.2
10	7.4§	11.7	4.3‡
11	7.7	11.6	3.9
12	8.1	11.4	3.3
13	8.2	11.7	3.5
14	7.7	11.5	3.8
15	7.9	11.7	3.8
16	8.2	11.4	3.2
17	8.4	11.2	2.8§
18	8.2	11.2	3.0
Averages	7.99	11.48	3.49

* Anesthetized with phenobarbital.
† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 8.6 μ, is smaller by 2.3 μ than the smallest diameter, 10.9 μ, recorded for a plugged vessel. The two series of measurements do not overlap.
‡ Largest.
§ Smallest.

TABLE 12.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Five Cats*: Measurements Made During Patent and Plugged Condition upon Different Vessels*

Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference in Average of Patent and Plugged Vessels, μ
8.4‡	11.6	
8.4	11.4	
8.0	11.5	
8.0	11.0§	
7.8	11.8	
8.1	12.0‡	
8.4	11.6	
8.1	12.0	
7.8	11.2	
8.2	11.5	
7.7§	11.6	
8.1	11.8	
8.4	11.0	
7.8	11.8	
8.2	11.7	
7.9	11.6	
8.0	11.3	
8.2	11.4	
7.7	11.6	
8.4	11.2	
8.0	11.4	
8.0	11.6	
7.8	11.2	
7.8	11.8	
8.2	11.6	
8.4	11.5	
7.8		
8.2		
7.8		
8.0		
8.4		
8.2		
8.08	11.52	3.44 (Averages from Tables 11 and 12)

* Anesthetized with phenobarbital.
† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 8.4 μ, is smaller by 2.6 μ than the smallest diameter, 11.0 μ, recorded for a plugged vessel. The two series of measurements do not overlap.
‡ Largest.
§ Smallest.

TABLE 13.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Five Dogs*: Measurements Made During Patent and Plugged Condition upon Same Vessel*

Vessel No.	Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference, μ
1	6.5‡	10.0‡	3.5
2	6.8§	10.2	3.4‡
3	6.8	10.4§	3.6§
Averages	6.7	10.2	3.5

* Anesthetized with phenobarbital.

† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 6.8 μ, is smaller by 3.2 μ than the smallest diameter, 10.0 μ, recorded for a plugged vessel. The two series of measurements do not overlap.

There are only three sets of measurements in this series because of difficulty in holding the tissue still. Note that the maximum, minimum, and averages fall within the range of similar figures recorded in Table 14.

‡ Smallest.

§ Largest.

TABLE 14.—*Internal Diameters of Precapillary Bottlenecks of Mesenteric Arterioles in Five Dogs*: Measurements Made During Patent and Plugged Condition upon Different Vessels*

Patent Internal Diameters, μ†	Plugged Internal Diameters, μ†	Difference in Average of Patent and Plugged Vessels, μ
6.6	9.9	
6.1‡	10.2	
6.9	10.4	
6.6	9.8	
6.5	10.0	
6.4	10.3	
6.7	9.9	
6.4	10.2	
6.8	10.0	
6.6	10.4§	
6.5	10.0	
6.4	9.8	
6.8	10.5	
7.0	10.2	
6.8	9.8	
7.0	10.0	
7.1§	9.8	
6.6	10.2	
6.8	10.2	
7.0	10.0	
6.8	9.8	
6.4	10.0	
6.6	10.0	
6.6	10.1	
6.4	9.9	
6.5	9.7‡	
6.4		
6.5		
6.6		
6.8		
6.5		
6.4		
6.6		
6.64	10.06	4.62 (Averages from Tables 13 and 14)

* Anesthetized with phenobarbital.

† These measurements are accurate to the nearest 0.5 μ. Note that the largest diameter recorded for a patent vessel, 7.1 μ, is smaller by 2.6 μ than the smallest diameter, 9.7 μ, recorded for a plugged vessel. The two series of measurements do not overlap.

‡ Smallest.

§ Largest.

Note that statistical treatment of the above data in terms of standard deviation, etc., was not necessary for the following two reasons: 1. The differences between the maximum diameter of a patent vessel in each species and the minimum diameter of a plugged vessel in the same species is considerably greater than the 0.5 μ error of the method of measurement. 2. At no point in the data is the maximum diameter of a patent vessel as great as the minimum diameter of a plugged vessel. The following tabulation summarizes this:

	Maximum Internal Diameter of Patent Vessels	Minimum Internal Diameter of Plugged Vessels
Frogs	16.9	19.1
Hamsters	6.6	8.8
Mice	6.8	8.1
Rat	6.4	7.7
Rabbit	5.9	7.2
Cat	8.6	10.9
Dog	7.1	9.7

TABLE 15.—*Summary of Minimum, Maximum, and Average Diameters*

Anesthetized, But Otherwise Untreated, Animals, Opened by Bloodless, Sludgeless Laparotomy	Internal Diameters of Narrowest Portions (Bottlenecks) of Patent Transilluminated Mesenteric Terminal Arterioles While Conducting Unagglutinated Blood			Internal Diameters of Transilluminated Mesenteric Bottlenecks Immediately After Embolization		
	Maximum	Minimum	Average	Maximum	Minimum	Average
Frogs						
Same vessel	16.9	15.3	16.2	20.6	19.2	20.0
Different vessel	16.9	14.9	16.1	21.6	19.1	20.2
Hamsters						
Same vessel	6.4	6.0	6.2	9.5	8.8	9.1
Different vessel	6.6	6.0	6.3	9.7	8.6	9.3
Mice						
Same vessel	6.6	5.2	5.7	9.6	8.1	8.7
Different vessel	6.8	5.2	5.9	9.7	8.7	8.9
Rats						
Same vessel	6.2	5.6	5.95	8.6	7.8	8.20
Different vessel	6.4	5.2	6.01	8.8	7.7	8.16
Rabbits						
Same vessel	5.8	5.2	5.52	8.4	7.7	8.07
Different vessel	5.9	5.0	5.50	8.2	7.2	8.06
Cats						
Same vessel	8.6	7.4	7.99	11.8	10.9	11.48
Different vessel	8.4	7.7	8.08	12.0	11.0	11.5
Dogs						
Same vessel	6.8	6.5	6.7	10.4	10.0	10.2
Different vessel	7.1	6.1	6.64	10.4	9.7	10.06

ABSTRACTION OF IDEAS FROM THE RECORDED DATA

There are certain concepts which can be taken directly from the data. These may be arrived at by pointing out the maxima, minima, and averages of the recorded internal diameters of the bottlenecks of the mesenteric vessels for each species examined, followed by calculations of the probable cross-section areas of the patent and the plugged bottlenecks.

From these calculations, it is possible to state the difference between the cross-section areas of the patent vessels and the cross-section areas of the corresponding plugged vessels. It is possible also to state the maximal degree of dilatation which, under the conditions of these experiments, the mesenteric bottlenecks did achieve as they were embolized.

Table 15, abstracted from the data, summarizes the maximal, minimal, and average diameters of terminal mesenteric arterioles of several species both before and after embolization.

Probable cross-section areas of patent and of embolized terminal arteriole bottlenecks were calculated.

As the data are accurate to but 0.5 μ, the calculations of cross-section areas presented below were made using but one significant figure.¶

It was assumed that the bottlenecks were circular in cross section. It is probable that most of these bottlenecks were circular, or very nearly circular, in cross section because (*a*) the pressure in the vessels probably was much higher than that of the tissues and fluids surrounding the vessels, and (*b*) had the bottlenecks deviated very far from circles, say toward ellipses, then some of the measurements should have recorded short diameters of the ellipses, and others, long. Such deviations would have appeared as widespread figures in the tables of data. The close grouping of the figures of each class in the tables of data, thus, is one form of evidence indicating that these vessels were nearly circular in cross section. Because our data

TABLE 16.—*Summary of Calculations*

	Rounded-Off Average Internal Diameters of Patent Bottlenecks, μ	Rounded-Off Average Internal Diameters of Plugged Bottlenecks, μ	Calculated Average Cross-Section Area of Lumen of Patent Bottlenecks, Sq. μ	Calculated Average Cross-Section Areas of Lumen of Plugged Bottlenecks, Sq. μ	Ratio of "Area After Plugging" to "Area When Patent"
Frogs	16	20	200	310	1.6
Hamsters	6	9	32	72	2.2
Mice	6	9	28	63	2.2
Rats	6	8	28	52	1.9
Rabbits	6	8	23	50	2.1
Cats	8	11	50	100	2.0
Dogs	6	10	30	78	2.2

were accurate to but one significant figure, the value of π (the ratio of the circumference of a circle to its diameter) was taken as 3.1 for these calculations.

Table 16 summarizes these calculations.

Summarizing the calculations on the ratios of the cross-section areas of the plugged and patent bottlenecks, we found these ratios were as follows: frogs, 1.6; hamsters, 2.2; mice, 2.2; rats, 1.9; rabbits, 2.1; cats, 2.0, and dogs, 2.2. It is interesting to note that the figures for all the mammals studied are remarkably similar.

If these numbers are examined in terms of significant figures, it becomes obvious that the terminal mesenteric arterioles of the above mammalian species became plugged when they were forcibly dilated to two times their own patent cross-section areas.

COMMENT

There is an interesting correlation between the diameters of the red blood cells of each of the mammals and the maximum diameters of the plugged bottlenecks which were measured. In the article on "The Erythrocytes," by Isaacs,[85] there are charts listing the measured diameters of the red blood cells of several different species, some measured wet, suspended in plasma or other fluids, and some measured after they were dried in smears. On comparison of the diameters of

¶ For a description of the correct mathematical use of significant figures, see Mode.[110]

the red blood cells reported by Isaacs with the diameter of the plugged precapillary bottlenecks, it becomes obvious that the diameters of the plugged precapillary bottlenecks are (as close as these measurements and calculations make it possible to estimate) approximately twice the diameter of the animal's own red blood cells. This holds for the six mammals measured.

If, by any chance, this relationship holds in human beings, then human precapillary bottlenecks can be expected to plug when dilated to somewhere between 15 and 19 or 20 μ.

The frog red blood cell is an ellipse. These usually are 14, 15, or 16 μ wide by 20, 21, or 22 μ long. In unagglutinated blood, frog red blood cells usually go through the precapillary bottlenecks longitudinally, or approximately longitudinally, and to some degree rolled or folded up (Krogh [1929][89]).

It has been observed repeatedly in our laboratory that basic masses larger and more rigid than those which plug the terminal precapillary bottlenecks always plug the arterial system farther upstream than the narrowest part of the bottleneck. Whenever one of these larger, more rigid masses enters a vessel whose internal diameter is greater than the mass, the mass is carried on downstream until the sides of the mass impinge upon the internal surface of the progressively narrowing, cone-shaped artery. After that the mass is driven downstream into and impacted in the artery.

Thus, in order to circulate, a sludge must consist of basic masses smaller than the smallest masses which will embolize precapillary sphincters.

It is indeed amazing that in the mammalian species we have examined, terminal arterioles systematically become plugged by solid masses whose diameters are no, or not much, larger than twice the diameter of the red blood cells of the species. Many similar measurements made in tissues and organs other than mesenteries are now needed.

As previously noted, all measurements recorded above were made in the terminal arteriole bottlenecks of mesenteric vessels. The damages which minute emboli do to the body are consequent upon the particular tissue or organ which receives the emboli. It might be possible to assume that the terminal arterioles of each and every organ of a given species have the same internal diameters under a given set of physiologic or pathologic conditions and that these terminal arterioles are equally distensible in each and every organ. However, these may not be safe assumptions. It is much more important, for the moment, to point out that the internal diameters and the degrees of distensibility may be quite unequal from organ to organ.

Knisely (1940),[91] after studying many transilluminated internal organs with microscopes, pointed out that each organ has its own pattern of blood vessels in space and that the vascular apparatus of each organ has its own set of contraction and relaxation patterns in time (Knisely, Bloch, and Warner [1948] [96]).

Thus, the measurements recorded from mesenteries above can be but a beginning toward direct measurements of portions of living vascular systems, measurements which should be made under varieties of conditions of health, disease, anesthesia (including the use of different anesthetics), and therapy. In order to facilitate the beginnings of such studies it seems wise to point out that a considerable background of information concerning the vascular patterns of individual organs already exists and that portions of many different organs have been or can be transilluminated

for microscopic study. Obviously, those structures which can be studied with microscopes during life can also be measured. Such measurements will be most useful in dealing with problems of the biophysics of disease.

A partial list of tissues and organs which can or ought to be so studied and parts measured follows. An introductory bibliography for each is given. No attempt has been made to make any reference list complete. Each reference is put in because it seems to introduce some aspect of such studies.

Arteriovenous Anastomoses.—Arteriovenous anastomoses have been seen in numbers of different organs. Probably they do not exist in every tissue and organ. There have been very few direct measurements made of the diameters of arteriovenous anastomoses during life under specifically defined physiologic or pathologic conditions. Indeed, there have been very few attempts made even to count the numbers of arteriovenous anastomoses per unit volume of tissue in individual organs. An excellent introduction to the literature describing arteriovenous anastomoses can be obtained from Grant (1930),[61] Grant and Bland (1930),[62] Modell (1933),[111] Clark (1938),[21] Boyd (1939),[22] Clara (1939),[50] Prinzmetal and co-workers (1947),[124] Prinzmetal and co-workers (1948),[123] Simkin and co-workers (1948),[138] and Tobin and Zariquiey (1953).[146]

The A–V Capillary.—Since 1939 a considerable literature has developed around the concept of the "through capillary," or a–v capillary, or "perferential channel." These structures have in general been seen in very thin tissues, which can easily be transilluminated for microscopic study. However, their presence has not yet been demonstrated in statistically valid samples of the series of tissues and organs of which the body is composed. Even so, complicated concepts of the hemodynamics of shock have developed, based in part upon assumption of the presence of a–v capillaries in significant numbers throughout or in major portions of the body.

A part of the concept of the functioning of these structures is based upon the transformation of pressure energy to kinetic energy and of kinetic energy to pressure energy, as described in Bernoulli's Theorem.

White (1924) [165] gives us a careful mathematical statement of Bernoulli's Theorem and the limits of its applicability to capillary beds.

During our generation, an intensive search will undoubtedly be carried out for the a–v capillary in various organs. Such research should include studies of its structure, function, and failure to function. An introduction to the literature dealing with these can be obtained from the references which follow: Zweifach (1939),[175] Chambers and Zweifach (1944),[27] Zweifach and co-workers (1945),[176] Chambers and Zweifach (1946 and 1947),# Chambers (1948),* Schmidt (1952),[134] and Grafflin and Bagley (1953).[59]

Bone Marrow.—Bone marrow is beyond our current ability to study microscopically during life under completely healthy and untraumatized conditions. However, the dimensions of portions of the blood vessels within bone marrows ought to be obtained. The vascular system of bone marrow has been studied by Doan (1922) [40] and Drinker and co-workers (1922).[41]

References 28, 29, and 8.

* References 25, 26, and 4.

Central and Peripheral Nervous Systems.—Determinations of the actual dimensions of the terminal arterioles within each and every portion of the central and peripheral nervous systems are greatly to be desired. The literature relating to such studies obviously is enormous, but the following references will give an introduction: Cobb and Talbott (1927),[32] Roofe (1935), [130] Bloch (1941 and 1943),† Scharrer (1944),[132] Scheinker (1947),[133] Wolf (1947),[174] Durward (1948),[42] Heymans (1950),[76] Opitz and Schneider (1950),[117] Krogh (1950),[100] Harvey and Rasmussen (1951),‡ Kenk and Nall (1952),[87] Hicks and Warren.[75a]

Heart.—It is impossible to know what degree of success, if any, might await attempts to study microscopically the circulation in superficial blood vessels of the mammalian myocardium while the heart is beating. The technical problem is to see very clearly something which is moving. The problem might not be as difficult as it seems at first. The heart rate could be slowed down, and the respiratory motions could be stopped by the continuous insufflation of oxygen into the trachea.

A surprising feature of some living organs is that the movements they make are completely cyclical in space. This feature may help a great deal in attempts to study the heart. Some years ago, members of this laboratory focused microscopes on the superficial layers of the myocardium of the beating hearts of frogs to see whether the contracting muscle fibers expanded enough to squeeze the blood capillaries between them sufficiently to stop the blood flow in the capillaries. The particular procedure which made many sharp, clear observations possible consisted in focusing the microscope at a given position of the heart beat cycle at which the heart stood still for a moment. By pressing slightly against one or more parts of the animal's body, it was possible to have this moment of physical arrest occur during several different portions of the cardiac cycle. Thus, the conditions of the vessels and blood in the myocardium were observed in sharp focus many times at each of the successive phases of the cardiac cycle, but no microscope field was held in focus during a single complete cardiac cycle. The observations under the conditions of these experiments were that the blood flow was stopped in the capillaries of the frog myocardium during each and every systolic phase of the cardiac contraction. The blood flowed in torrents through these vessels during the diastolic portions of the cycle.

It seems probable that very high speed motion pictures, taken through the microscope, would make it possible to record portions of such cycles and to measure the diameters of the vessels.

If the shortening, thickening muscle fibers of the myocardium of the mammalian heart compress the vessels between them as tightly as occurred in the frog hearts observed, then one would expect that during the flow of agglutinated blood through the smallest cardiac vessels of sick mammals and man the rhythmic compressing action would provide an extra force acting to crush the masses of sludge in the smallest vessels. Of course, we now should very much like to know what kinds and degrees of damage are done to the linings of these small vessels by being rhythmically crushed against the sides of passing masses of tightly agglutinated blood cells.

† References 20 and 21.

‡ References 70 and 71.

Other methods of studying beating hearts in vivo and cardiac vessels are reported by Estable and Vaz Ferreira (1931),§ Gramenitzki (1934),[60] Barta (1936),[4] Vonwiller and Semenov (1936),[149] Prinzmetal and co-workers (1947),[124] and Simkin and co-workers (1948).[139]

Pituitary.—The vascular apparatus of the pituitary gland has been studied by investigators by a variety of methods. The vessels of the pituitary gland and its stalk are known to be complicated and certainly deserve direct study with measurements of the parts under defined physiologic and pathologic conditions. It will not be easy to devise satisfactory surgical approaches, but if the surgical approaches can be worked out, the studies ought to be very much worth while (Herring [1908],[74] Dandy and Goetsch [1911],[26] Popa and Fielding [1930 and 1933],‖ Basir [1932],[6] Cushing [1933],[25] 'Espinasse [1933],[48] Houssay, Biossati, and Sammantino [1935],[81] Collin [1936],[22] Wislocki and King [1936],[173] Stevens [1937],[143] Wislocki [1937 and 1938],¶ Morato [1939],[112] Green [1947, 1948 and 1951],# Green and Harris [1949],[66] and McConnell [1953] [109]).

Kidney.—Since the observations of Richards and Schmidt (1924),[127] it has been possible to transilluminate for microscopic study portions of the kidney of amphibians. Walker and Oliver (1941) [157] have made such studies possible in mammals. Knisely, Bloch, and Warner (1948) [96] measured the diameters of the afferent and efferent arterioles of frog kidney glomeruli under various experimental conditions. Warner (1949) [159] observed the glomeruli of Amphiuma tridactylum as they became plugged with masses of agglutinated blood cells. She recorded her observations by taking motion pictures of this process through the microscope.

Thus, it is now possible to transilluminate and measure portions of living glomeruli of amphibians and mammals during health and disease.

An introduction to the literature describing observations on living kidney glomeruli can be obtained from the following references: Richards and Schmidt (1924),[127] White (1924 and 1928),* Wearn and Richards (1924),[162] White and Schmitt (1925),[167] Hayman (1927),[72] Bensley (1929),[11] Richards (1929),[126] Bieter (1930),[14] Ekehorn (1931),[43] Berglund and co-workers (1935),[13] Adolph (1936),[1] Ellinger (1940),[44] Walker and Oliver (1941),[157] Walker and co-workers (1941),[158] Selkurt (1945 and 1946),† Knisely and co-workers (1948),[96] Warner (1949),[159] Smith (1951),[140] Grafflin and Bagley (1952),[58] and Hartman (1954).[67a]

Liver.—Within recent years microscopic study of living liver lobules of various species has become technically possible, in mammals as well as in frogs. The techniques of intratracheal insufflation of oxygen worked out by Dr. John Irwin has made it possible to hold the liver of mammals very still for high magnification microscopic study (Irwin and Macdonald [1953] [84]). The following list includes a high percentage of the articles which have appeared so far on the microscopic study of living livers: Löffler and Nordmann (1925),[104] Hartoch (1931),[68] Dietrich (1932),[39] Franke and Sylla (1933 and 1934),‡ Knisely (1939),[90] Bloch (1940),[17] Wakim

§ References 46 to 48.
‖ References 120 and 122.
¶ References 170 to 172.
# References 63 through 65.
* References 165 and 166.
† References 135 and 136.
‡ References 54 and 55.

(1941, 1942, 1944, and 1946),§ Wakim and Mann (1942 and 1944),‖ Knisely and co-workers (1948),[96] Seneviratne (1949),[127] Burrage and Irwin (1953),[24] and Irwin and Macdonald (1953).[84]

Lung.—Living frog and mammalian lungs can also be studied at high magnifications with direct measurements of the diameters of portions of the living histology (Hall [1925],[67] Olkon and Joannides [1930],[115] Tiemann and Röder [1932],[145] MacGregor [1933],[105] Wearn and associates [1934],[161] Prinzmetal and co-workers [1948],[123] Burrage and Irwin [1953],[24] Tobin and Zariquiey [1953],[146] and Irwin and Burrage [1953] [83]).

Ovary.—The blood vessels of the ovary change during different phases of reproductive activities. Almost certainly, ovarian vessels could be observed and measured during life (Bassett [1943];[7] Delson, Lubin, and Reynolds [1948];[38] Nalbandov and James [1949],[113] and Williams [1948] [168]).

Pancreas.—The islands of Langerhans of the pancreas have a very specialized pattern of blood vessels. Each island has its own blood supply, just as does each kidney glomerulus. It is now quite feasible to study and to measure the dimensions of the vascular apparatus of the pancreatic islands (Bensley [1915],[10] Van Glahn and Choboi [1925],[148] Warren and Root [1925],[160] Covell [1928],[34] O'Leary [1930],[114] Berg [1930],[12] Beck and Berg [1931],[8] Hirsch [1931 and 1932],¶ Wharton [1932],[164] Ries [1935] [126]).

Spleen.—Microscopic study of living mammalian spleens has been carried out within recent years. Peck and Hoerr (1951) [118] present drawings made with an accuracy equal to those made with a good camera lucida. From their drawings the actual dimensions of various portions of the splenic vascular apparatus can be obtained. Although it is well known that infarcts of the spleen are a most common lesion of this organ, we do not yet know how small an embolus can initiate an infarct. Recent studies of the spleen have been done by Knisley (1936),# Mackenzie and co-workers (1941),[106] Snook (1944), [141] Utterback (1944),[147] Björkman (1947),[16] Williams (1950),[169] Peck and Hoerr (1951),* Eguchi,[42a] Gelin,[56a] McNee,[106a] Nakata and co-workers,† Knisely,[97a] Osgoe,[117a] and Palm.‡

Striated Muscles.—The blood vessels of whole small muscles can be studied while the muscles are at rest, during contractions, and at different phases of the post-contraction hyperemia (Bischoff and Ricker [1932] [15]; Knisely, Bloch, and Warner [1948] [96]).

Adrenal Gland.—It should be possible to make microscopic observations of the adrenal glands of small mammals, such as bats and small mice. The adrenal glands of frogs are beautiful, have a tremendous blood supply, and are very easily studied. Descriptions of adrenal blood vessels have been given by Flint (1900),[53] Bennett and Kilham (1940),[9] and Gersh and Grollman (1941).[57]

§ References 150 through 154.

‖ References 155 and 156.

¶ References 76 thorugh 79.

References 88 and 89.

* References 118 and 119.

† References 112a-112e.

‡ Palm, P. T.: A Microscopic Study of the Circulatory System of the Living Trans-illuminated Spleen of the Mouse and Rat, thesis submitted to the Graduate School of the University of Pittsburgh, 1951.

Thyroid Gland.—Portions of the living thyroid gland can be transilluminated for microscopic study. The thyroid has a large blood supply which varies greatly under different conditions. In the dog (Modell [1933] [111]) the gland has many apparently large arteriovenous anastomoses. Detailed studies of the vascular apparatus of the mammalian thyroid gland during health and disease, with physical measurements of the parts, seem long overdue (Hartoch [1932],[69] Modell [1933],[111] Rochlina [1936],[129] Legait [1949][102]). Algire and Uhlenhuth [2a] publish a technique for cytological study.

Tumors.—The vascular patterns, reactions and dimensions of small tumors can be studied, particularly those which are transplantable (Algire and Legallais [1951] [2]).

Uterus.—The vascular system of the uterus certainly changes dimensions greatly during different phases of the estrus or menstrual cycle and during pregnancy. Determination of the diameters of terminal vessels under various conditions ought to be possible (Markee [1932 and 1940],§ Daron [1936],[37] Bartelmez [1937],[5] Holden [1939],[80] Bacsich [1941],[3] Jacobsen [1944],[86] and Williams [1948] [168]).

Vasa Vasorum.—Vasa vasorum, the small blood vessels which nourish the walls of the large blood vessels, are necessary for the preservation of the normal healthy anatomy of the walls of the large vessels. It should be possible to devise methods for studying such small vessels and measuring them (*a*) while healthy normal blood is flowing through and (*b*) while the small vessels are conducting agglutinated blood. In spite of the fact that the walls of large blood vessels are known to undergo extensive histological modifications during arteriosclerosis, we do not yet know the sizes of the smallest masses which will directly embolize vasa vasorum, thereby causing infarcts in the walls of large vessels. O'Neill (1947) [116] showed that blood flow through vasa vasorum was absolutely necessary in order to preserve the healthy anatomy of the large vessels. From time to time, when studying living internal organs under the microscope, one comes across easily visible vasa vasorum. Methods for systematically studying and measuring these certainly should now be worked out (Ramsey [1936],[125] O'Neill [1947],[116] and Samuels and co-workers [1952] [131]).

Portion of the internal ear, particularly the spiral ligament and stria vascularis of living guinea pigs, can now be transilluminated for microscopic study and measurement (Weille and co-workers [1953] [163]).

Studies on the living vascular systems of portions of experimental animals such as those outlined above can perhaps be extended to similar studies of parts of human beings.

Devices should be constructed and opportunities sought for making some of the above measurements on various parts of living human beings. Almost certainly measurements could be made by using high magnification microscopes focused on the bulbar conjunctiva of a man (*a*) before anesthesia, (*b*) after the anesthetic but before surgery, and, again, (*c*) following surgical repair of, say, a hernia.

Bloch (1953) [18] has made measurements of the sizes of masses which have embolized terminal arterioles of human bulbar conjunctiva during acute poliomyelitis.

§ References 107 and 108.

Further, measurements should be made on the diameters of these bottlenecks in human beings suffering from a variety of diseases. It is quite possible that in some as yet unknown diseases there may be, in response to the disease, some type of permanent enlargement of precapillary bottlenecks either all over the body or in selected organs.

It also seems necessary that such measurements should be made after treatment of animals with drugs which cause maximal relaxation of the bottlenecks. It would seem that there must be a maximal effect which vasodilator drugs could possibly produce. In other words, vasodilator drugs might help (in dilating vessels so that sludged blood could pass through) to some finite but limited degree.

In order to find the maximum possible degree of dilatation, it might be wise to make such measurements on vessels before and after surgical removal of the regional sympathetic ganglia, which are a major factor in the control of vasoconstrictor tonus of small vessels.

At present, it seems inescapable that these arteriole bottlenecks set one finite physical limit on the life of each vertebrate individual.

SUMMARY

During health the circulating red cells of animals and man are not agglutinated but tend to repel each other slightly.

Intravascular agglutination of the circulating blood or sludged blood, has been found to exist and be a part of the pathologic physiology of a number of diseases and pathologic states of animals and of man.

Geometrically, arterioles and terminal arterioles are segments of long, slowly tapering, truncated cones.

Terminal arterioles and other portions of peripheral vascular bottlenecks have a limited degree of distensibility.

In some pathologic conditions, the blood in the arterial system, including that passing down terminal arterioles, is agglutinated.

The masses of agglutinated blood cells making up a sludge may be divided into two separate classes, basic masses and charge aggregates. The basic masses are by definition those which do not break up into smaller parts as they are forced into or through tapering terminal arterioles and capillaries.

Two properties of each basic mass, its diameter and its degree of internal rigidity, operating simultaneously, determine the resistance which the mass presents to being pushed down the progressively narrowing terminal arteriole.

As a basic mass, which is sufficiently large and rigid to embolize a terminal arteriole, is carried down and forced into the terminal arteriole, the mass forcibly bulges that vessel before it plugs, impacts, or embolizes the vessel. Hence, the firmly embolized vessel has a somewhat greater internal diameter immediately after embolization and impaction than it had before.

Minute emboli, both sterile and infected, are, of course, well known to morphologic pathologists. But the actual dimensions of masses which are just too large to pass through terminal arterioles of any species have not been known. Thus, one phase of the problems presented by sludged blood is to determine the maximal size which the narrowest part of the terminal arteriole bottlenecks can attain before or as each becomes plugged with one or more masses of sludge.

A new type of ocular micrometer, which permits measurements to be made of living blood vessels, is introduced in this paper. The micrometer does not touch the living tissue, and with it measurements can be made, limited only by the accuracy of the lens system of the microscope.

A series of measurements was made in mesentery to determine the internal diameters of the terminal arteriole precapillary sphincters when the terminal arterioles had a patent lumen with unagglutinated blood passing through. These measurements were made in the mesenteries of several species, namely, frogs, hamsters, mice, rats, rabbits, cats, and dogs.

Another series of measurements was made to determine the internal diameter to which these terminal arteriole precapillary sphincters dilate as each becomes plugged with a mass of agglutinating blood cells. These measurements were made in the same species listed above.

Taking into account "significant figures," we found that the average internal diameter of plugged mesenteric vessels were for frogs 20 μ, for hamsters 9 μ, for mice 9 μ, for rats 8 μ, for rabbits 8 μ, for cats 11 μ, and for dogs 10 μ.

The cross-section areas of these mesenteric vessels before plugging were calculated, and the cross-section areas after these vessels were forcibly bulged as they became impacted with small emboli were also calculated.

The ratios of the cross-section areas of the plugged bottlenecks to the cross-section areas of the patent bottlenecks were for frogs 1.6 μ. Similarly, for hamsters, mice, rats, rabbits, cats, and dogs the mesenteric arterioles became solidly impacted when the terminal arterioles had a cross-section area but two times the areas which they had had in the relaxed state.

As noted, the above measurements and calculations were all derived from studies of mesenteric vessels. There is no reason to assume that the diameters and degrees of distensibility of terminal arterioles are the same in every organ of a single animal or species. It is now possible to study living transilluminated portions of a series of other tissues and organs. The diameters of terminal arterioles in each of these ought to be determined both while the vessels are relaxed and as they become impacted. An introductory bibliography is given for the literature describing studies of the vascular system of each of a series of organs.

In order to circulate, a sludge must consist of basic masses smaller than the smallest masses which will embolize precapillary sphincters.

At present, the conclusion seems inescapable that these arteriole bottlenecks set one finite physical limit on the life of each individual, and of each vertebrate species.

Mr. A. W. Strickler, of Chicago designed and constructed the ocular micrometer herein described.

Mr. Strickler is a member of the London Horological Society, an association devoted to the study of mechanisms which measure and record time. Members of this Association have designed and constructed some of the most precise chronometers which have ever been made. Mr. Strickler called upon a lifetime of scholarly study and craftsmanship for the construction of the instrument.

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CYTOPLASMIC RIBONUCLEIC ACID IN THE HUMAN LIVER CELL

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THE BASOPHILIC property of cytoplasmic ribonucleic acid (RNA) has been employed as an experimental tool in the study of this important cellular component.[1] Extensive investigations of RNA have been made in the liver cells of experimental animals under the influences of starvation and during injury and regeneration.* Several papers have appeared in which these histological techniques have been used in the study of the cytoplasmic RNA of the human liver cell.†

This report deals with the cytoplasmic RNA of the human liver cell, as studied by basic-dye staining methods and by ultraviolet spectrophotometric techniques.[12] Differences in the state of cytoplasmic RNA have been demonstrated between the freshly fixed cell obtained by biopsy and the partially autolyzed cell obtained at postmortem.[12]

METHODS

The data were derived from 100 biopsy (12 surgical, 88 needle) and 131 postmortem specimens of human liver. The following fixatives were employed: Zenker's, Carnoy's, and Bouin's solutions, absolute and 95% ethyl alcohol, acetone, and 10% formalin. Specimens were embedded in paraffin, sectioned at 5 to 10 μ, and stained with a variety of basic dyes: Giemsa, azur I, azur II, toluidin blue, methylene blue, and pyronin. The ribonuclease experiments were performed under the following conditions: 0.2 mg. per cubic centimeter of enzyme in distilled water without buffer (approximately pH 6) at 50 C. for two hours. The trichloroacetic acid (TCA) extractions were performed with a concentration of 5% TCA for 15 minutes at 85 C. The ultraviolet spectrophotometric studies were performed by the methods of Caspersson[14] as modified by Pollister and Ris[15] and Pollister.[12] The absorption curves were measured with the apparatus shown in Figure 1, equipped with reflecting optics.

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* References 2-7.

† References 8-11.

RESULTS

Biopsy Specimens.—When liver cells of normal, well-fed rats were stained with basic dyes, heavy coarse clumps of basophilic material were seen in the cytoplasm, against a fairly clear background (Fig. 2). These clumps could be digested with ribonuclease, extracted with hot trichloroacetic acid, and they absorbed ultraviolet light at 2,600 A. Previous treatment with lanthanum acetate or chloride rendered these clumps resistant to the action of ribonuclease. The nuclei demonstrated basophilic material which could be identified as both RNA and deoxyribonucleic acid (DNA) by treatment with ribonuclease, deoxyribonuclease, and Feulgen staining.

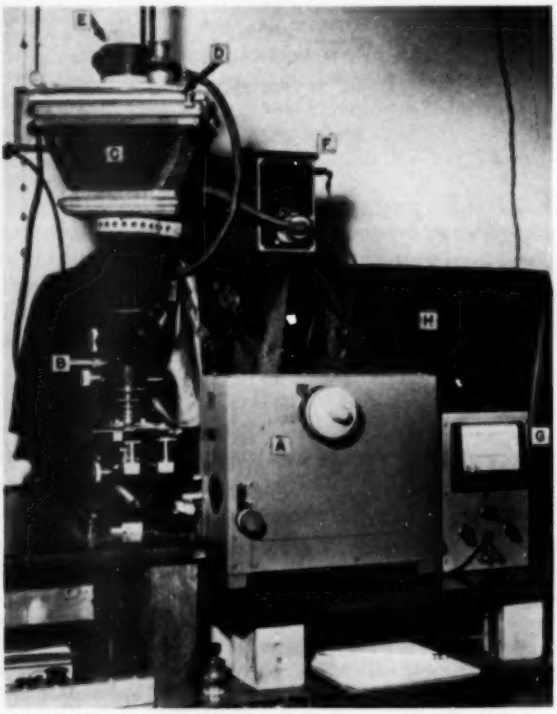

Fig. 1.—Microspectrophotometer for measuring absorption curves of small regions of a cell. Major components are (*A*) grating monochromator (Bausch and Lomb), (*B*) microscope with side observation tube (E. Leitz), (*C*) photomicrographic camera (C. Zeiss), (*D*) rotating-plate type of photometer mount,[11] (*E*) photomultiplier, (*F*) photometer with IP 21 tube and battery power supply (Farrand Optical Co.) modified for use with the (*G*) vacuum tube type of microammeter with six sensitive ranges (RCA, model WV84A), and (*H*) light source, which is beneath the black curtain. (For ultraviolet absorption curves reflecting optics are used, and the source is a small hydrogen discharge tube with stabilized power supply [National Technical Laboratories].)

When the human liver biopsy specimen was stained with basic dyes, the cytoplasm usually showed an interlacing of faintly basophilic, finely granular, and fibrillar material. The background was clear and very lightly basophilic. The nuclei contained basophilic material, most of which could be identified as DNA by Feulgen staining and by deoxyribonuclease treatment (Fig. 3).

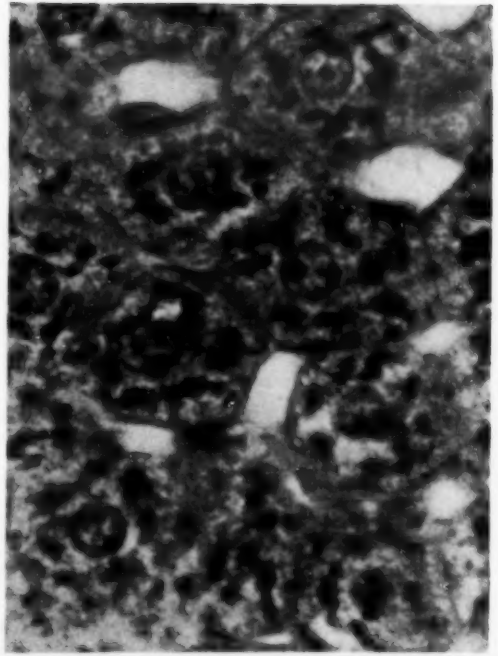

Fig. 2.—Normal rat liver. The coarse basophilic granules are seen prominently in the cytoplasm. Nuclear basophilia is almost obscured by the heavy cytoplasmic basophilia. Oil immersion, 5 μ, fixed in Carnoy's solution, Giemsa stain; × 1,200.

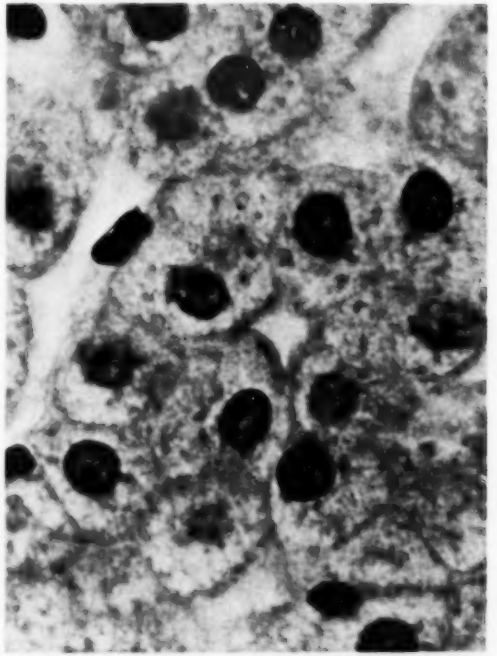

Fig. 3.—Human liver (biopsy).—The lightly basophilic granules and delicately interlacing material in the cytoplasm are less prominent than the nuclear basophilia. The appearance contrasts strikingly with that of liver cells in Figures 2 and 5. The tissue was obtained from a healthy adult male. Oil immersion, 5 μ, fixed in Carnoy's solution, Giemsa stain; × 1,200.

The previous treatment of the biopsy tissue with lanthanum salts was without effect upon staining properties. The digestion of the biopsy specimens with ribonuclease did not materially alter the staining characteristics. Previous digestion with amylase or trypsin did not change the cytoplasmic basophilia, although amylase digestion removed all stainable glycogen. Whether the specimen was from a normal liver or a diseased liver (hemachromatosis, Laennec's cirrhosis, hepatitis, obstructive jaundice) did not seem to alter the cytoplasmic basophilia. Fatty cells showed relatively little staining with dye because much of the cytoplasm was filled with fat droplets.

The biopsy specimens from two healthy subjects were studied by the ultraviolet spectrophotometric methods of Caspersson [14] as modified by Pollister and Ris [15] and Pollister.[12] These studies showed that treatment with ribonuclease did not change the extinction coefficients and therefore did not digest RNA. On the other hand, hot trichloroacetic acid treatment reduced these values markedly, showing that cytoplasmic RNA had been removed (See Table).

Effects of Ribonuclease and Hot Trichloroacetic Acid Treatments upon the Ultraviolet Absorption of the Human Liver Cell (Biopsy)

Treatment	No. of Observations	Mean E_{280}	Standard Error of Mean
Case I			
Untreated	65	0.388	0.003
Ribonuclease control *	40	0.372	0.003
Ribonuclease †	40	0.204	0.004
Hot TCA ‡	50	0.175	0.007
Case II			
Untreated	64	0.425	0.004
Ribonuclease †	35	0.410	0.005
Hot TCA ‡	20	0.190	0.005

* Distilled water, pH 6.0, at 50 C. for two hours.
† 0.2 mg. enzyme per cubic centimeter in distilled water without buffer, pH 6.0, at 50 C. for two hours.
‡ 5% TCA (trichloroacetic acid), at 85 C. for 15 minutes.

This phenomenon was then investigated further by studying the ultraviolet absorption of the cytoplasm at different wave lengths, from 2,300 to 3,300 A. A typical curve (Fig. 4) showed two distinct peaks, one at 2,600 A., representing RNA, and one at 2,750 A., representing protein. After ribonuclease digestion the curve was unchanged. However, after extraction with hot trichloroacetic acid the nucleic acid peak at 2,600 A. was gone but the protein peak at 2,750 A. remained. The curve obtained by subtraction has its peak at 2,600 A. and is the curve for RNA. These findings indicated that the cytoplasmic RNA of the freshly fixed human liver cell was in a state in which it did not combine strikingly with basic dyes nor was it digested with ribonuclease. However, it absorbed ultraviolet light at the expected wave lengths and it was extractable with hot trichloroacetic acid.

Postmortem Specimens.—The postmortem specimens were fixed and stained in the same manner as the biopsy specimens. In many instances discrete coarse clumps of cytoplasmic basophilic material were demonstrated, similar to those in the rat liver cell (Fig. 5). In some specimens the coarse clumping was replaced by a more finely granular basophilic deposit, and in general, material obtained more

than 24 hours postmortem showed less of the coarsely clumped basophilia and more of the finely granular material. These two types of basophilia did not seem to be related to the pathological state of the liver, although in the few instances of massive hepatic necrosis, there was no basophilic clumping. The hepatic cells from many normal livers showed fine basophilic granularity, and those from many cirrhotic livers showed well-preserved coarse basophilic clumps. Both types of basophilia could be digested with ribonuclease and could be extracted with hot trichloroacetic acid.

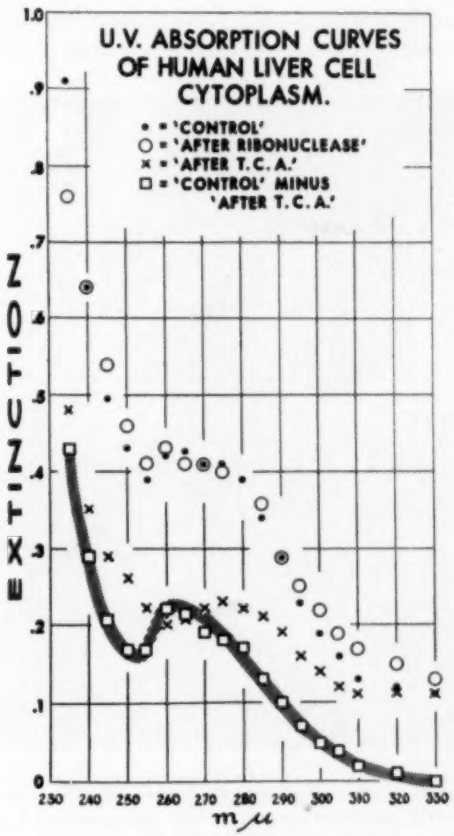

Fig. 4.—Ultraviolet absorption curves of human liver cell cytoplasm (biopsy). The "control" and "after ribonuclease" curves are identical, indicating no digestion of RNA by the enzyme. The "after TCA" curve is markedly depressed and has its peak at 2,750 A., representing the absorption peak for protein. The "'control' minus 'after TCA'" curve peaks at 2,600 A. and represents the absorption peak for the RNA which has been extracted by TCA. The measurements were made through unstained sections, 3 μ in thickness and mounted in glycerine.

The basophilic cytoplasmic pigment in the cells about the central veins has been described in both biopsy and postmortem specimens and is not nucleic acid.[16]

Because of the variable staining properties in many of the postmortem specimens, studies were made of the effects of in vitro autolysis upon cytoplasmic basophilia.

Specimens were incubated at 4 and 37 C. in serum and saline for one to eight hours. The results showed that with continuing autolysis there was progressive dissolution of the coarse basophilic clumps and that after two hours only finely granular basophilic material remained. The cells showed marked shrinkage at this time, similar to that seen in many postmortem specimens. The results were the same in all these experiments.

The differences between the staining properties of the cytoplasmic RNA in freshly fixed biopsy tissue and in postmortem tissue suggested that autolysis in postmortem tissue might liberate RNA for combination with basic dyes. Accordingly, biopsy tissue was incubated at 37 C. for 4, 12, and 24 hours in serum and

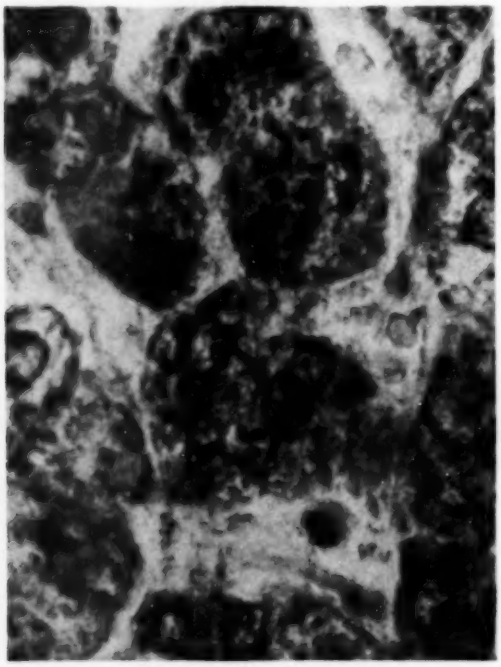

Fig. 5.—Human liver (postmortem). The tissue was obtained from a patient who suffered a traumatic death. Postmortem examination was performed eight hours after death. The coarse basophilic granulation of the cytoplasm is similar to that seen in Figure 2, rat liver. Oil immersion, 5 μ, fixed in Carnoy's solution, Giemsa stain; × 1,200.

saline. Coarse basophilic clumps were not observed in any of the specimens. There was progressive shrinkage with dissolution of many cells, most marked at 24 hours. Whatever the role autolysis may have in liberating RNA, it could not be duplicated in vitro.

COMMENT

The histochemical demonstration of various cellular components has become a widely employed cytological technique. However, before basing physiological interpretations upon results obtained by such methods, it is well to have criteria for

their acceptance. The cytoplasmic RNA in the rat liver cell is basophilic, is digestible with ribonuclease, is extractable with hot trichloroacetic acid, absorbs ultraviolet light at 2,650 A., and is precipitated by lanthanum salts. In tissues other than the rat liver, some workers have accepted basophilia with reduction of intensity after ribonuclease digestion as proof of identity as RNA. Some have even relied upon basophilia alone. However, it is noteworthy that cytoplasmic basophilia may be related to the presence of protein and some loss of basophilia may be nonspecific, following enzyme digestion.

Even when all of these criteria are met, the quantitative estimate of the presence of RNA by histochemical means is still a hazardous one. It has not been established that the basophilia of RNA can be considered in the relatively quantitative manner applicable to DNA, as demonstrated cytochemically by the Feulgen technique. Indeed, RNA may be present in over 60% of its normal amount without cytoplasmic basophilia (e. g., fasted rat). Furthermore, it is likely that RNA may exist in necrotic cells with eosinophilic cytoplasm. This deduction is derived from data on CCl_4-poisoned rats. Twenty-four hours after a single parenteral injection of CCl_4, one-half of each liver lobule may be necrotic and filled with cells with eosinophilic cytoplasm. The cytoplasm of the remaining cells may be filled with small vacuoles and contain no discrete basophilic clumps. Yet such a liver may have normal levels of RNA per unit dry or wet weight as determined chemically.‡ These data indicate that much RNA may be present without having its usually ascribed basophilic properties.

The present studies were undertaken with the expectation that the basic-dye staining methods might be used for the quantitative estimation of RNA. The results show that this is not feasible. Indeed, the state of RNA in freshly fixed human liver cells differs from that in postmortem human and rat liver cells. In its resistance to staining it resembles the nucleus of the living cell.[17]

The staining of RNA in postmortem tissue is unpredictable and seems to be related to factors which are not only beyond experimental control, but which are not specific for any disease state. The elapsed time between the death of the patient and the fixation of the tissue is an important factor. The longer this interval, the less the likelihood of finding stained RNA clumps. It is possible, on the basis of evidence derived from experimental animals, that the nutritional state of the patient is another important factor. Other incidental and nonspecific factors may be fever and hypermetabolism as preterminal events. Popper has described certain agonal morphological changes which occur in human liver cells.[18] These variables seem of sufficient importance to invalidate the results of histochemical studies (with basic dyes) of hepatic cellular RNA in postmortem tissue.

With regard to the freshly fixed liver cell, McKay and Farrar [9] noted that cytoplasmic RNA was stained with difficulty. They attributed this finding to the presence of glycogen in these cells. By contrast, in postmortem liver cells where RNA could be demonstrated, glycogen was absent. In the present experiments, the glycogen of biopsy specimens was removed by amylase digestion and the tissue was stained subsequently with basic dyes. No differences were noted in cytoplasmic basophilia before and after this treatment. The control amylase digestion of post-

‡ Reference 7 and unpublished data.

mortem material did not alter its cytoplasmic basophilia. Therefore it seems doubtful that the presence of glycogen in biopsy tissue is responsible for the difference in RNA staining.

The results of these studies demonstrate that RNA in the freshly fixed human liver cell is in a physicochemical state different from that of the postmortem cell. In the biopsy tissue, combination with the basic dyes is not striking. Furthermore, from the experiments with ultraviolet spectrophotometry, it can be concluded that ribonuclease digestion of RNA does not occur. In the biopsy specimen, RNA may exist in a complex combination with protein or lipid or as a higher polymer. In this state hot trichloroacetic acid may split RNA from such linkages and extract it. With the death of the cell, RNA combines with basic dyes and is digested by ribonuclease. The factors responsible for this transformation are not clear. This process could not be reproduced by in vitro autolysis of biopsy tissue. As autolysis progresses, stainable RNA disappears from the postmortem specimen. Further evidence for the presence of RNA in a state which does not combine with basic dyes is obtained in the liver cells of fasted and CCl_4-poisoned animals, where considerable amounts of RNA may be present without appreciable cytoplasmic basophilia.

From the data presented, it appears that the use of basic dyes for the estimation of cytoplasmic RNA can not be applied to the study of the human liver cell.

CONCLUSIONS

1. The cytoplasmic RNA of the freshly fixed human liver cell does not stain as coarse basophilic clumps, nor is it digested by ribonuclease. It may be extracted from the cell with hot trichloroacetic acid.

2. The cytoplasmic RNA of the postmortem human liver cell may stain well with basic dyes, is digested by ribonuclease, and is extracted with hot trichloroacetic acid. The roles of certain nonspecific factors in the study of postmortem tissues are discussed.

3. The use of basic staining for the estimation of cytoplasmic RNA cannot be applied to the study of the human liver cell.

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DURAL NODULES IN RHEUMATOID ARTHRITIS

Report of a Case

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THIS IS thought to be the first reported recognition of dural rheumatoid nodules. In a necropsy upon a patient with advanced rheumatoid arthritis, typical rheumatoid nodules were found in the cranial dura, as well as in the heart, pericardium, sclera, splenic capsule, lungs, esophagus, skeletal muscle, and a peripheral nerve. In 1819, Scudamore [1] noted that rheumatoid arthritis was essentially a disease of the "fibrous textures of the body." This observation has increased in significance as it has been applied to periarticular changes and to widespread extra-articular lesions.

The ocular manifestations of rheumatoid arthritis, also present in my case, were described by Van der Hoeve [2] in 1931 and designated scleromalacia perforans. Approximately 30 examples * of this uncommon manifestation of rheumatoid arthritis have been recorded.

Freund and his co-workers † reported rheumatoid nodules in perineurium and in skeletal muscles not related to joints. Cardiac, pericardial, and perivascular lesions have been reported.‡ Baggenstoss and Rosenberg [21] in 1944 described lesions in the heart which resembled the subcutaneous nodules, and similar lesions have been reported by Graef, Hickey, and Altmann [22] in 1949 and by Sokoloff in 1953.[23] There are reports of lesions in the lungs, pleura, and peritoneum. However, most accounts lack specific evidence of rhematoid origin or do not include microscopic findings.§

REPORT OF CASE

Mrs. M. E., a 64-year-old white housewife, had had rheumatoid arthritis for 21 years. Cortisone had been administered by her physician but was discontinued when glycosuria and decreased urinary output were noted. Under butazolidine therapy, anemia progressed and the erythrocyte count dropped to 2,600,000 per cubic millimeter. She was given three transfusions. Scleral ulcers had been present for four years, and for them cortisone and sulfanilamide eye drops had been used. Recently the patient had noted dyspnea and edema of the ankles. While she was bedridden, a large sacral decubital lesion developed. The arthritic symptoms had increased three weeks before her admission to the University Hospital, on Aug. 11, 1953.

By physical examination at admission the patient was found to be chronically ill and to have marked rheumatoid deformities of the hands, wrists, elbows, knees, and ankles. The blood pressure was 90/70; pulse, 138 per minute apical and 120 per minute radial; respirations, 20 per

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* References 3 to 14.

† References 15 and 16.

‡ References 6 and 17 to 20.

§ References 6, 17, and 24 to 26.

minute, and oral temperature, 97.8 F. The ophthalmologist stated that severe scleromalacia perforans (rheumatoid scleritis) was present, with a scleral ulcer 1 mm. in diameter, O.D., and staphyloma of the choroid, O.D. superiorly. There was apparent cardiomegaly, and auricular fibrillation was believed to be occurring. Electrocardiograms revealed small QRS complexes in all leads and questionable auricular fibrillation, evidence of which was absent in later tracings. Pericardial effusion was noted, and subsequently a pericardial friction rub developed. The sacral decubital ulcer measured 8 by 8 cm. Marked pitting edema of the back and legs was present. Nodular pulmonary lesions, shown by roentgenograms, which were interpreted as tuberculous, and a globular heart shadow were noted. A skeletal survey showed marked generalized osteoporosis in addition to the rheumatoid arthritic deformities. The hemoglobin level was 9.7 gm., and the leucocyte count was 10,000 per cubic millimeter.

A consultant from the arthritis unit of the internal medicine service concurred in the diagnosis of advanced rheumatoid arthritis. The patient was placed on a program of digitoxin, mercaptomerin (Thiomerin),‖ and hydrocortisone eye drops. Wet dressings were used for the decubital ulcer, and the patient was placed on a Stryker frame preparatory to skin grafting. Pericardial aspirations were performed on Aug. 18 and 19, and 200 cc. of fluid was obtained at the second procedure. Direct microscopic examination of this fluid revealed many polymorphonuclear leucocytes and a few lymphocytes. The fluid was cultured for tubercle bacilli and pyogenic organisms, but no growth occurred.

The patient died on Aug. 20.

Necropsy Findings

External findings at necropsy were essentially the same as at admission.

On removal of the calvarium, firm white nodules were noted in the dura. Each nodule was about 2 mm. in diameter and slightly elevated above the inner surface of the dura. In some places these nodules had coalesced to form granulomatous plaques up to 5 cm. in diameter. These lesions were generalized and appeared to have no relationship to blood vessels. Nodular involvement of the basilar dura was no more marked than that elsewhere. On removal of the dura from the cerebrum only slight adherence to the pia-arachnoid was present. No exudate was noted in the subarachnoid space. The nodules were opaque to transmitted light. On sectioning, the smaller discrete lesions were homogeneous, but the plaques were slightly softened in the center. The arachnoid granulations were of the usual size and appearance. The sinuses, veins, and arteries appeared to be normal.

The brain weighed only 1,170 gm., and the convolutions were slightly flattened and sulci somewhat less distinct than normal. On section no evidences of inflammation, vascular disease, or neoplasm were noted.

In the pericardial sac, 100 cc. of cloudy fluid was found and all surfaces showed fibrinous exudate. The heart weighed 280 gm., and brown atrophy of the myocardium was noted. The base of each aortic valve cusp was thickened.

Fibrinous pleuritis and parenchymal pulmonary nodules were present. The latter measured approximately 1 cm. in diameter, and some of them had necrotic centers. Similar foci were noted in the bronchial lymph nodes. Nodules were present in the splenic capsule but were not observed in the parenchyma. The wall of the gall bladder was thickened, and mixed pigment and cholesterol calculi were present. No lesions were noted in the liver, pancreas, or gastrointestinal tract. The kidneys showed arteriosclerotic nephropathy and papillary necrosis. Adrenal cortical lipids were depleted. There was polypoid hyperplasia of the endometrium. Tissue specimens of synovial membrane were secured from the left knee and elbow joints. Permission to remove the eyes was granted.

Histopathologic Findings

The dural nodules showed central fibrinoid necrosis with a bordering zone of epithelioid cells arranged as a palisade. Peripheral to the epithelioid zone there was a third region, containing polymorphonuclear leucocytes, lymphocytes, plasma cells, histiocytes, and giant cells of the Touton type. In some areas the necrotic

‖ Wyeth Laboratories, Philadelphia.

foci became confluent. In all respects these nodules resembled the lesions recognized as rheumatoid when in juxta-articular and subcutaneous positions. No inflammatory process was present in the inner meninges or brain.

The heart showed nodules, similar to those in the dura and considered to be rheumatoid, in the annulus fibrosa near the base of the valves, adjacent to a coronary artery, and in thickened pericardium. Fibrinous pericarditis, moderate coronary atherosclerosis, a marked increase in the pigment at the poles of the myocardial nuclei, and degenerative fatty infiltration of the myocardium were present.

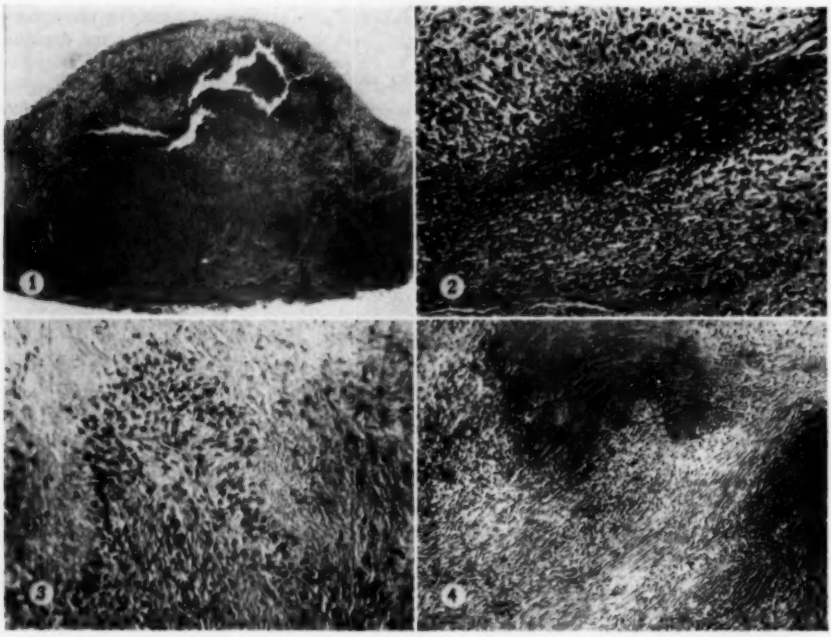

Fig. 1.—Large rheumatoid nodule projecting from inner surface of dura. Necrotic state of interior is evident from unavoidable artifacts resulting from manipulation and fixation. Hemalum and eosin; × 30.

Fig. 2.—Small rheumatoid nodule in dura, with characteristic zonal organization. Hemalum and eosin; × 105.

Fig. 3.—Characteristic palisading of nuclei around area of fibrinoid necrosis in dura. Hemalum and eosin; × 111.

Fig. 4.—Conglomerate rheumatoid nodule in dura. Hemalum and eosin; × 51.

In the lungs there were two large nodules which were probably rheumatoid and, in addition, an area of old caseation necrosis which appeared to have eroded into a small bronchus. No active tubercles were noted. Anthracosilicosis was the principal pathologic process in the bronchial lymph nodes. Hyalinized areas of anthracosilicosis and necrotic rheumatoid nodules were found in these lymph nodes.

Rheumatoid nodules were present in the thickened splenic capsule. No hepatic parenchymal nodules were present. Chronic cholecystitis and cholelithiasis were

found. Adrenal cortical lipids were depleted. The kidneys showed arteriosclerotic nephropathy and papillary necrosis. Osteomalacia, with subperiosteal bone reabsorption, was present in the vertebrae and tibiae. The bone marrow was 50 to 60% cellular. Marked polypoid hyperplasia of the endometrium was present.

A subcutaneous nodule from the left elbow consisted chiefly of hyalinized scar tissue, giant cells, and areas of necrosis with cholesterol clefts. Presumably this was originally a subcutaneous rheumatoid nodule. The cholesterol could have resulted either from old hemorrhage in the necrotic center (although no traces of blood pigment could be seen) or from necrosis of adipose tissue.

Both eyes showed extensive involvement of the entire sclera with typical rheumatoid nodules which frequently become confluent. The inflammatory process did not involve the cornea or uveal tract but began abruptly at the limbus and extended to the margins of the optic nerve. A rheumatoid nodule was present also in a small nerve near the optic nerve, probably a ciliary nerve, and another nodule was found in an extraocular muscle.

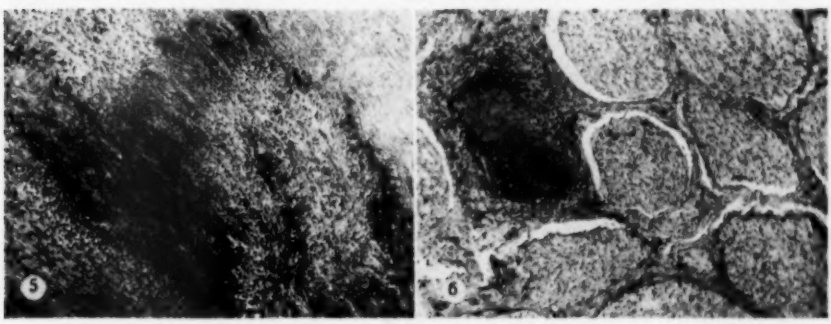

Fig. 5.—Portion of periphery of large conglomerate rheumatoid nodule in sclera. Hemalum and eosin; × 69.

Fig. 6.—Small rheumatoid nodule in perineurium of ciliary nerve. Hemalum and eosin; × 66.

The tissue removed from the knee joint consisted of hyalinized scar tissue covering the articular surface. A few epithelioid and giant cells with scattered wandering cells were present.

COMMENT

The nodules in this case were typically rheumatoid in structure and cannot be attributed to tuberculosis. The patient gave no history of tuberculosis, and acid-fast stains revealed no tubercle bacilli. The lesions were not distributed as would be expected in tuberculosis. The absence of parenchymal tuberculous involvement of the liver, spleen, bone marrow, kidneys, and inner meninges would be unusual with miliary tuberculosis which involved such unusual structures as the dura, eyes, and heart. The histologic features of the nodules were unlike those of true tubercles. It has been noted in the literature that the necrosis in the rheumatoid nodule is not complete and fragments of intact collagen remain in the areas of necrosis.[21] The caseation necrosis of tuberculosis is complete. The inner zone of palisading epithelioid cells and outer zone of inflammation are unlike those seen in a typical tubercle.

That the nodules were due to syphilis is extremely unlikely. No history of syphilis was obtained, the lesions were not characteristically gummatous, and the blood serologic test was negative. Histoplasma capsulatum was searched for and not found.

The dural lesions were histologically like those described in the literature and as seen in this laboratory from other extra-articular situations in patients with rheumatoid arthritis. The peripheral neural, subcutaneous, and ocular involvement are accepted manifestations of this disease. We have seen similar pericardial lesions in other patients who have had rheumatoid arthritis.

SUMMARY

In a patient with rheumatoid arthritis, dural rheumatoid nodules were numerous and form the subject of this report. The sources consulted have shown no description of such dural involvement.

This case provides further evidence that rheumatoid arthritis is a disseminated disease involving many organs in addition to the usual articular and periarticular regions.

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RECENT DEVELOPMENTS IN ENVIRONMENTAL CANCER

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National Cancer Institute, National Institutes of Health, Public Health Service, and Department of Health, Education and Welfare.

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I. OCCUPATIONAL CANCERS TWENTY-FIVE YEARS AGO

SOME TWENTY-FIVE years ago the total factual knowledge on environmental cancer from all sources was limited to information on cancers of the skin from occupational, environmental, and/or medicinal contact with solar radiation, roentgen and radium radiation, arsenicals, coal tar, and petroleum distillates. The excessive frequency of bladder cancer among Egyptian farmers infected with Schistosoma hematobium and among aniline dye manufacturers exposed to some, at that time still unidentified, aromatic amines had been noted. Information also was available on the habitual cancers of the lips and oral cavity in pipe smokers and betel quid chewers, as well as of the cancers of the abdominal skin among users of certain heating devices, known as kangri or kairo.

Not only was the total number of environmental carcinogens and cancers recorded in the United States before 1925 very small, but some of the occupational cancers, such as arsenic cancer of the skin and aniline cancer of the bladder, observed in Europe had not yet made their appearance in this country (Table 1).

Nothing was known at that time concerning the established or strongly suspected carcinogenic action of nickel, chromium compounds, arsenicals, and asbestos upon the lung and/or nasal cavity and sinuses or of a similar effect by coal tar fumes, mineral oil mists, and isopropyl oil vapors. No information existed on the leukemiogenic sequelae of low-level exposures to benzene, roentgen radiation, and radiation

from radioactive substances. Although the development of occupational osteogenic sarcoma following the ingestion of radioactive material was soon to be discovered, the recognition of other cancerous complications resulting from excessive medicinal exposures of the bones to roentgen or radium radiation was still two decades away (Table 2).

TABLE 1.—*Occupational Cancer Cases Recorded in the United States Before and After 1928**

Agent	Sites of Cancer	Number of Cancers in U. S. A.		Number of Cancers in Europe	
		Up to 1928	Up to 1952	Up to 1928	Up to 1952
Arsenic	Skin	..	7	14	25
Coal tar pitch	Skin	6	110	908	3,150
Petroleum	Skin	50	70	26	29
Shale oils	Skin	..	7	435	1,900
Lignite tar; paraffin	Skin	..	..	14	14
Creosote oil	Skin	..	1	26	40
Anthracene oil	Skin	..	..	19	20
Soot; carbon black	Skin	..	1	189?	<190
Aromatic amines	Bladder	..	250-300	220	1,100-1,200
Roentgen radiation; radium radiation	Skin	30	?	68	?
	Leukemia	1	16	5	13
	Lung	..	..	311	650
Totals		98	450-500	2,235	7,250

* The numbers of cases listed for the various occupational cancers represent, in part, estimates based on unpublished information. However, in all groups the numbers given are lower and, presumably, much lower than the actual number of cancers attributable to the occupational carcinogens mentioned. The data available did not permit making any kind of a guess of the number of cancers caused by an occupational exposure to solar radiation or associated with chronic schistosomiasis of the bladder.

TABLE 2.—*Occupational Carcinogens and Cancers Discovered During the Last Twenty-Five Years (1928-1952)*

Agent	Site of Cancer	Type of Exposure	Number of Recorded Cases*		
			U. S. A.	Other Countries	Total
Arsenic	Lung	Respiratory	7	16	23
Asbestos	Lung	Respiratory	12	47	59
Chromates	Lung	Respiratory	75	65	140
Nickel	Lung;	Respiratory	0	84	84
	nasal cavity; nasal sinus		0	51	51
Coal tar; pitch	Lung	Respiratory	0	53	53
Petroleum	Lung	Respiratory	7	33	40
Benzene	Leukemia	Respiratory	11	31	42
Isopropyl oil	Lung;	Respiratory	1	0	1
	larynx;		4	0	4
	nasal sinus		6	0	6
Radioactive chemicals	Bones;	Oral	9	0	9
	nasopharynx		3	0	3
Total			130	380	510

* The numbers of cases listed for the various occupational cancers represent close approximations of the recorded or known evidence. They do not reflect the actual number of cancers attributable to the different occupational carcinogens because of the highly defective and incomplete records available.

Some two decades ago information and, therefore, concern was still lacking about potential human cancer hazards from exposure to a large number of chemicals with which, for some reason, occupational and general population groups have established contacts, and which have so far elicited only in experimental animals can-

TABLE 3.—*Potential Environmental Human Carcinogens*

Agent	Site of Tumor	Type of Exposure	Species	Types of Human Contacts
Estrogens, natural; synthetic	Breast Lymph nodes Uterus Hypophysis	Parenteral Cutaneous Oral	Mouse Rat Rabbit Guinea pig	Manufacture Dietary additive Cosmetic ingredient
Carbon tetrachloride	Liver	Oral	Mouse	Industrial solvent Degreasing agent Dry cleaning agent Fire extinguishing agent Manufacture Production of freon Grain fumigant Extractive of oils
Chloroform	Liver	Oral Parenteral	Mouse	Manufacture Anesthetic Solvent and extractive of oils, resins, rubber, waxes, iodine, alkaloids Ingredient of lacquer, floor polish, cleaning fluid Production of artificial silk, plastics
DDT	Liver	Oral	Rat	Manufacture Insecticide Food contaminant
Tannic acid	Liver	Parenteral	Rat	Ingredient of foodstuffs (fruits, wine, coffee, and tea) Medicinal agent Tanning agent
Thiourea and derivatives....	Liver Thyroid	Oral	Rat	Citrus fruit preservative Medicinal agent
Dulcin	Liver	Oral	Rat	Manufacture Sweetening agent
Diethylene glycol	Bladder	Oral	Rat	Antifreeze Intermediate in explosive manufacture Softener of lacquer, inks, wood stains, glue, textiles Humectant, tobacco
Methylated naphthalenes	Skin	Cutaneous	Mouse	Vehicle of insecticides
Bergius coal oils, tars.....	Skin Subcutaneous tissue	Cutaneous Parenteral	Mouse Rat	Manufacture Fuel Lubricants Plastic production Petrochemical
Fischer-Tropsch coal oils, tars, waxes, greases	Liver	Cutaneous Parenteral	Mouse	Manufacture Fuel Lubricant Petrochemical
Light green S. F. ..... Brilliant blue F. C. F. Fast green F. C. F.	Subcutaneous tissue	Parenteral	Rat	Textile dyes Food and cosmetic dyes
Butter yellow	Liver	Oral	Mouse Rat	Food dye (Orient) Gasoline dye
Cellophane ..... Polyethylene Polyamide Bakelite	Subcutaneous	Parenteral	Rat Mouse	Film, fiber, plastic-industrial manufacture and uses Wrapping material Medicinal agent
Beryllium	Bone	Parenteral Respiratory	Rabbit Rat	Metal alloy, x-ray tube, phosphor manufacture Refractory vessels Atomic energy production
Selenium	Liver Thyroid	Oral	Rat	Soil contaminant Coloring matter of glass, ceramics, paint, rubber Metal alloys Rubber accelerator Photoelectric apparatus Decolorizer Fireproofing agent Medicinal agent

cers in various organs. While some of these chemicals existed but had not yet been tested for carcinogenic properties at that time, others had not been discovered and, therefore, had not become a part of the human environment providing occupational, medicinal, dietary, cosmetic, or other environmental contacts. Among these potential human carcinogens are several carbon polymers used as plastics, films, fibers, adhesives, and lacquers, namely: cellophane, polyethylene, and polyvinyl chloride. To this group also belong the metals beryllium and selenium, and the chlorinated hydrocarbons, chloroform, carbon tetrachloride, and dichloro-diphenyl-trichloroethane (DDT), and also diethylene glycol, methylated naphthalenes, the sweetening agent dulcin, synthetic estrogens, urethane, thiourea, and several aromatic azo and amino compounds including three certified food dyes. Nothing was known 25 years ago about the neurofibromatogenic action of ergot and the hepatoma-producing effect of the alkaloid of red pepper when fed to rats. We still had to learn that certain dietary deficiencies might have causal relations to cancers of the thyroid, oropharynx, and liver (Table 3).

TABLE 4.—*Carcinogenic Chemicals Eliminated As Environmental Human Carcinogens*

Agent	Site of Cancer	Type of Exposure	Species	Intended Use
Styryl 430	Subcutaneous tissue	Parenteral	Mouse	Trypanocidal agent
2-Acetylaminofluorene	Subcutaneous tissue Bladder Liver Brain Thyroid Acoustic duct Breast Leukemia Kidney Lung	Oral Parenteral	Mouse Rat Dog Fowl	Insecticide

To this list of potential environmental human carcinogens discovered during the last two decades may be added several aromatic compounds which were originally developed for medicinal or pesticidal purposes (styryl 430, trypanocidal agent,[1] and 2-acetylaminofluorene, insecticide[2]) and which were discarded for such uses when, fortunately, chronic toxicity tests revealed their strong carcinogenic properties. The dye, *p*-dimethylaminoazobenzene (butter yellow), also belongs to this group, since its use as a food dye was prohibited in this country on toxic grounds in 1919, although its potentially more important carcinogenic properties were not demonstrated until 1936[3] (Table 4).

Mankind, thus, was fortunate in having been spared a possibly extensive exposure of several highly carcinogenic environmental poisons by the accidental disclosure of such qualities during chronic toxicity tests.

It was, thus, no wonder that 25 years ago the general interest in and awareness of the existence of environmental and occupational cancers and carcinogens by the medical profession, public health agencies, and industrial management was of a distinctly low order. Even the few known industrial carcinogens received at that time little, if any, attention. In fact, the very existence of some of these carcinogens, such as the carcinogenic coal tars and pitches and petroleum distillates, seemed to be forgotten for many years. Their carcinogenic properties were only recently rediscovered. Cancer in industry, 25 years ago, was almost exclusively acute traumatic

cancer and, for that matter, apparently still is, as far as workmen's compensation boards and accident insurance companies [4] are concerned, despite the fact that there are on record in this country an impressive number of environmental and occupational cancers attributable to diverse chemical and physical agents. It appears that most of the compensation claims advanced in such matters are settled without the aid and knowledge of official agencies.

II. RECENT DEVELOPMENTS IN OCCUPATIONAL AND ENVIRONMENTAL CANCERS

With the rapid increase in factual information on occupational and environmental carcinogenic agents during recent decades, there has come a rapidly growing increase in the awareness and appreciation of these dangerous attributes of our new artificial industrial environment. This development is strikingly illustrated by the fact that during the last five years active interest in occupational cancer problems has been shown by a considerable number of individual industrial concerns as well as entire industrial groups (manufacturing, processing, and consuming industries of aniline dyes and antioxidants, chromates, nickel, beryllium, arsenic, coal tar and pitches, petroleum, carbon black, isopropyl alcohol, rubber, tobacco, uranium ores and radioactive substances and devices, copper-arsenic ores, and asbestos). Some concern about such matters has been evinced also by commercial producers and/or users of estrogens, benzene, selenium, and various pesticides.

The enumeration of these various industries and products places in distinct perspective the known and potential environmental cancer problem as related to the spectrum of recognized and suspected environmental carcinogens, the various occupational worker groups involved, and the consumer and general population groups actually or possibly affected. Through the impact of this evidence upon current medical thinking the subject of environmental and occupational cancer has formed a part, or has been the exclusive item, of the programs of many recent medical meetings of national and regional nature and of publications emanating from such gatherings. It has been discussed in magazines, over the radio, and on television for lay consumption. The epidemiologic and experimental study of environmental and occupational cancers and cancer hazards has become an integral part of the investigations of the National Cancer Institute and forms the subject of consideration of special subcommittees of the Committee on Growth of the National Research Council, of the Council on Industrial Health of the American Medical Association, and of the New York State Health and Labor Departments. The Food and Drug Administration of the Department of Health, Education, and Welfare has devoted an increasing amount of work to the study of the actual or potential cancer hazards to which producers and consumers of foodstuffs, medicines, and cosmetics may become exposed through the presence of carcinogenic additives and contaminants in these materials. Field studies of the Division of Occupational Health of the same department carried out during the last few years have dealt with cancer hazards related to the production of chromates and uranium ores. Several state health departments (California, New York, Ohio, Connecticut, Colorado, and New Jersey) engaged in special investigations concerning occupational cancer hazards to workers in industries of their respective states. With the aid of grants from the National Cancer Institute, special research organizations have been established during the last few years in three universities (University of Southern California, Los Angeles; University of Utah, Salt Lake City, and University of Pittsburgh, Pittsburgh) for

studying particularly the various aspects of air pollution with carcinogenic industrial and domestic waste products and the role which geologic and metereologic factors may play in determining the level and type of exposure of the general population to such environmental and industrial carcinogens.

The deliberations of the Delaney Committee of the House of Representatives Investigating the Use of Chemicals in Food Products (81st and 82d Congresses, 1951 and 1952) also were concerned with the possible and/or actual cancer hazards to the American people which might originate from such sources and which might require legislative attention.[5]

The developments of the last few years thus have not only enlarged our general knowledge on the environmental causes of human cancer but they have also laid a rational and sound basis for a future control of human cancer by preventive and prophylactic measures; i. e., for the elimination of cancer at its source.

III. CANCERS AS ENVIRONMENTAL DISEASES

The rapid growth of our factual information on the environmental causes of cancer, which has occurred particularly during the last 10 years, has consisted of data on general epidemiologic and demographic aspects of cancer as well as of evidence indicating the action of more or less well-defined and specific environmental agents in the production of cancer among members of restricted population groups. Although many of the most important observations made in these respects were reported from abroad, especially Great Britain, they are of direct value to this country because they are applicable with no or little modifications to conditions prevailing here. Their consideration and inclusion is, moreover, justified on the basis of the factually well-supported doctrine of a universal action of environmental carcinogens; i. e., environmental carcinogens when operating under similar conditions of exposure are equally effective regardless of their place or country of origin and irrespective of the particular type of contact (occupational, medicinal, cosmetic, dietary, or general environmental). Environmental carcinogens are, in general, as little concerned as the multitude of pathogenic microorganisms about the national, racial, and sociologic aspects under which they operate.

A. Demographic Data

Marked variations in the incidence of cancers of all sites and in their distribution by different sites have been demonstrated in population groups inhabiting different geographic regions, countries, and climates and belonging to different races.[6]

Cameron,[7] listing the cancer death rates per 100,000 population for 29 countries, noted a maximal rate of 176 for Switzerland, followed by a rate of 172.3 for England and Wales; by rates ranging between 165 to 120 in decreasing order for Austria, Scotland, Germany, Denmark, Netherlands, Norway, Sweden, France, and Ireland; by rates around 120 for New Zealand, the United States, and Belgium; by rates dropping from 117 to 101 for Canada, Australia, and Finland; by rates varying from 99 to 50 for Uruguay, Italy, Argentina, Japan, Chile, Spain, Brazil, and Greece, and by rates between 23 and 11 for Egypt, Mexico, Peru, and Ceylon. Without any doubt, a good part of these variations can readily be explained as due to differences in the completeness and reliability of recording cancer deaths in different countries as well as by the existence of very pronounced differences in the age composition of their populations. It may also be assumed that certain biologic factors

may play a role, although only a minor one, in bringing about such wide variations, which at their extremes (Switzerland and Ceylon) amount to a 16-fold gap in incidence rates. Since appreciable differences are noted, however, between cancer death rates of countries with very similar types of racial populations, medical care and biostatistical recording systems, it stands to reason that with a few exceptions regional environmental carcinogenic factors are mainly involved in bringing about these discrepancies.

This conclusion receives considerable support from the results of analytic study of the mortality and morbidity rates of cancers of different sites, such as the alimentary tract, liver, skin, penis, lung, and blood-forming organs (leukemia), among populations of various countries and members of different racial groups, especially those living in different geographic areas and habitats under different sociologic, dietary, and hygienic conditions, such as members of the white race living in Europe, America, South Africa, and Indonesia; Negroes living in Africa and America, and Chinese living in China, Indonesia, and America.*

1. *Cancer of the Stomach.*—A comparison of the total cancer mortality rates with the percentages of gastric cancer deaths for all cancers among males for coun-

TABLE 5.—*Cancer Mortality Rates for 100,000 Males and Percentages of Gastric Cancer Deaths*

Country	Cancer Mortality Rates	Gastric Cancer Percentage
England and Wales	118	22.8
United States	120	24.4
Netherlands	115	55.5
Bavaria	115	55.8
Norway	123	56.6
Sweden	120	60.5
Japan	...	53.3

tries of similar racial composition and medical and public health standards is most instructive in this respect (Table 5).†

The striking differences in the relative frequency of gastric cancer among cancers of all sites for the first six countries listed, having racially related populations, contrast sharply with the rather minor variations in their total cancer mortality rates. It is obvious, moreover, that Japanese males are as susceptible to gastric cancer as Dutch, Bavarian, Norwegian, and Swedish males. Liability to gastric cancer appears also to be pronounced for Chinese living in China [12] or in Indonesia ‡ where gastric cancer is infrequently found among the native Indonesians of Malay extraction.

Since in 1940 the Negro death rate in the United States from gastric cancer was reported as 14.4 for 100,000 as compared to 20.4 for the white population [15] it may appear upon first view that hereditary racial factors may be the determining influences in bringing about such discrepancies in gastric cancer death rates among two racially different population groups subject to apparently identical environmental factors. However, such a conclusion is not justified without first analyzing in detail

* References 8 and 9.

† References 10-29.

‡ References 13 and 14.

the living, working, and dietary habits of different racial groups coexisting in the same general environment. Wu and Loucks [12] thus noted that the high frequency of gastric cancer among Northern Chinese might be attributable to the possible presence of carcinogenic substances in certain grains used in the manufacture of a strong native drink (pai kan), of which fully 50% of the gastric cancer patients were habitual consumers.

2. *Cancer of Oropharynx.*—The necessity of a global study of apparently race-connected but actually environment-conditioned differences in cancer incidence becomes also evident from an analysis of the reputedly high racial incidence of nasopharyngeal cancers among Chinese, especially South Chinese.§ Martin [16] maintains that this distinct racial susceptibility to nasopharyngeal cancer is even found among orientals, some of them born in the United States, where nasopharyngeal cancer is much more common among white males than among white females (3:1).‖ There are, however, two observations which militate against a racial and genetic background of these lesions among Chinese. One is the fact that the racially related Japanese do not seem to share the peculiar susceptibility to nasopharyngeal cancer.[21] The second and more significant evidence against a racial factor is represented by the observation of a high frequency of oropharyngeal cancers among the female population living in northern Sweden, which is affected not infrequently by the Plummer-Vinson syndrome of chronic oropharyngitis possibly related to a chronic dietary deficiency of vitamin B complex factors and sideropenia.

It is noteworthy that the sex ratio of nasopharyngeal and oropharyngeal cancers of epidermoid type in the Swedish population is reversed (male to female ratio, 35:65) ¶ and that oral cancer also is more often found in Scandinavian women (43% of total number in Sweden, 32% in Denmark, 30.8% in Norway) than in women of other countries (5-25%).[29]

Since environmental cancers have a notoriously long latent period and because specific exposures to carcinogenic agents causing cancers occurring during adult life may have been sustained during childhood, the fact that Chinese afflicted by nasopharyngeal cancers lived in part in good economic conditions at the time when the cancers became manifest does not exclude the long-delayed carcinogenic action of environmental factors to which they may have become exposed during childhood. Thus, both demographic and sex distributions of nasopharyngeal and oropharyngeal cancers do not favor a race-connected causation of or susceptibility to these neoplasms, but suggest rather that environmental # and, most likely, dietary factors are involved in their production.[28]

3. *Cancer of Liver.*—A critical analysis of the information concerning the causes underlying the remarkable variations in the frequency of primary cancer of the liver in population groups inhabiting different geographic areas and having different racial origins provides evidence also favoring the environmental concept over the racial one. The bulk of the available data on this subject was recently recorded and analyzed by Berman [30] (Table 6). Additional evidence on the excessive frequency of primary liver cancer among the inhabitants of Algeria,[31] Senegal,[32] Sudan and

§ References 16-22.

‖ References 26 and 27.

¶ References 22 and 23.

References 26 and 27.

Southern Rhodesia,[33] Javanese and Indonesian Chinese,[34] Japanese,[35] and Koreans [36] supports the claim as to the existence of a primary liver cancer belt stretching from Africa through Indonesia into China and Japan.[36]

There can be no doubt that the excessive frequency of primary liver cancer among African Negroes, Indonesians, Chinese, and Japanese represents an abnormal incidence of these neoplasms and is not merely a phenomenon related to selective observations or the reflection of the age composition of the particular population groups. Berman estimates that the incidence of primary liver cancer among the South African Bantus is about 5 per 100,000 per annum, and among the Portuguese natives about 29 per 100,000. The relative frequency of primary carcinoma of the liver among American Negroes, on the other hand, is only moderately increased, according to autopsy evidence, over that of American whites (0.44% and 0.26%

TABLE 6.—*Frequency of Primary Cancers of the Liver in the Autopsy Material of Different Countries*

Country	Number of Autopsies	Liver Cancers	
		Percent of Autopsies	Percent of Cancers
Belgium	3,564	0.62	3.13
England	31,583	0.13	1.26
Germany	171,712	0.11	1.09
Holland	2,936	0.17	0.09
Hungary	6,000	0.30	....
Italy	19,008	0.23	....
Switzerland	13,250	0.29	1.56
United States	108,632	0.27	2.5
South Africa (Bantu)	5,864	1.2	50.0
China; Chinese in Malaya, Indonesia	23,764	0.9	33.0
Japan	15,565	0.97	7.5
Philippine Islands	13,876	0.44	22.2
India	14,768	0.32	17.5
Indonesia (Malayans)	8,253	1.3	41.6
East Africa (Uganda, Tanganyika, Kenya)	2,204	0.8	31.0
West and Central Africa (Congo, French Equatorial Africa, Fernando Po)	.....	....	17.2

respectively).* There exists, moreover, a marked shift toward young age groups for the liver cancer cases among the Bantus.† It is therefore most unlikely that a racial factor plays a major role in the demographic distribution of primary liver cancer.

This concept receives additional support from the fact that cirrhosis of the liver, which according to Hueper [45] has an established causal relation as a precursor condition to cancer of the liver, also is frequent among the population groups showing an excessive liability to cancer of the liver.‡ The significance of this relationship in regard to causal factors receives additional emphasis from numerous observations attesting the combined cirrhosis and cancer producing action upon the liver of various environmental agents (dietary deficiencies, azo dyes, seneca alkaloids, chlorinated hydrocarbons, tannic acid, selenium, infectious agents, and other hepatotoxic factors).

* References 30, 37, and 38.

† References 30, 43, and 44.

‡ References 32, 39-42.

4. *Cancer of the Skin.*—The incidence and topographical distribution of cancers of the skin, including melanomas, in various demographic and racial groups, provide another illustration of the greater importance of environmental carcinogenic factors over those of genetic origin.

The marked differences in the demographic and geographic distribution of skin cancers are strikingly illustrated by a table prepared by Hoffman [7] (Table 7).

Since these regional variations in skin cancer mortality cannot plausibly and justly be explained by differences in diagnostic proficiency and methods of death registration, it seems to be probable that they are primarily conditioned by local causal or contributory factors.[7] This conclusion receives some support from a study of the racial incidence of cutaneous cancers.

There exists reliable evidence indicating that nonmelanotic cancers of the skin are much more frequent among members of the white race than among Negroes living in Africa and in America § and among Indonesians.[34] During the period 1940 to 1945 the skin cancer mortality for American whites fluctuated between 2.6 and 2.9 for 100,000, while that for Negroes ranged from 0.7 to 0.9.|| Shapiro [56] reported from South Africa that 33% of all cancers found among white hospital

TABLE 7.—*Death Rates of Skin Cancer per 100,000 Population in 11 Countries**

Country	Rate	Country	Rate
United States	2.7	Switzerland	1.9
Ireland	2.7	Scotland	1.7
Australia	2.3	Holland	1.4
England; Wales	2.1	Uruguay	1.1
Cuba	2.0	Bavaria	0.8
		Japan	0.7

* From Hoffman, F. L.: The Mortality from Cancer Throughout the World, Newark, N. J., Prudential Insurance Company of America, 1915; cited by Cameron,[7] p. 20.

patients were skin cancers, while they represented only 8.4% in the Negro patient population. Corresponding morbidity figures from the United States noted skin cancers to be present in 20.9% of all white male cancer patients and in 11.0% of all white female cancer patients. These values contrast sharply with the much lower rates for both sexes in Negro patients (0.89 to 1.91%).¶

This preponderance of skin cancer among whites is almost entirely attributable to the relatively frequent involvement of the exposed parts of the skin (head, neck, forearm, and dorsum of hand 85 to 95%) and does not extend to cancers of the unexposed parts (trunk, leg, etc.).[52] Carcinoma of the exposed skin was the most frequently observed tumor among American war veterans[49] (17.1% of all cancer patients; 8.45% of all white patients with cancer, 0.6% of all Negro patients). In contrast, carcinoma of the covered skin was relatively rare in the white patient group (0.8% of all patients) but had a fairly high percentage among Negroes (9.78).

These differences in the topographical distribution of skin cancers among members of white and nonwhite races prevails irrespective of whether the members of the two racial groups reside in their normal habitat or are living in a foreign habitat (Indonesia, Africa).#

§ References 46-56 and 32.

|| References 46-48.

¶ References 49-51.

References 34, 49, and 50.

It is, moreover, remarkable that only about 0.5% of all cancers among whites involve the leg and the foot, while these parts are relatively frequently the site of cutaneous cancers in nonwhite inhabitants of the tropics (Africa, Indonesia, and Indochina about 30 to 45%),* These race-connected differences in the incidence and topographic distribution of skin cancer seem to be related exclusively to the protective action exerted against exogenous carcinogens by the increased pigmentation present in the skin of nonwhites, since Shapiro [56] found that albino Negroes exhibit a susceptibility to cancer of the exposed skin identical with that noted in whites.

Brief mention may be made of the fact that cancer of the lower lip also displays in its racial incidence a pattern similar to that of cancer of the skin by being more frequent among whites than among nonwhites.† Like cancers of the covered skin, cancers of the lip are more frequent in colored females (1.7% of all cancers) than in colored males (0.5%; corresponding figures for females and male whites, 7.3 and 10.2%, respectively).[49] Since general experience with the sex ratio of occupational cancers indicates that exposure conditions to exogenous carcinogens play an important role in determining the sex ratio, it can be concluded that such factors exercise an important influence not only on the sex ratio of lip cancer for the two racial groups but also on the incidence rates of these tumors among members of the two races.[63] Differences in pigmentation modify the action of the carcinogenic factor.[64]

The racial relations of melanomas, on the other hand, present a different picture. The American literature contains numerous references to the apparent rarity of malignant melanomas in Negroes in contrast to their relative frequency among American whites.‡ This has been ascribed to a better control of the pigment-forming tissue in the Negro.

However, from an analysis of hospital populations, it appears that the difference in malignant melanoma frequency between American whites and Negro patient populations is not very striking and might be attributable to a selective bias (Hewer [68] [Baltimore], white to Negro: 3.3:1; Anderson [69] [Memphis], 4.2:1; Morris and Horn [70] [Philadelphia], 4.4:1; Muelling and Burdette [71] [New Orleans], 2.1:1). This impression is supported by observations from Africa indicating that malignant melanomas among African Negroes are not particularly rare and that, for this reason, the existence of a race-conditioned resistance of the Negro race against melanomas cannot be assumed.§ Lee [72] thus asserted that Egyptian Negroes are 100 times as susceptible to melanomas as American Negroes and Shapiro noted that the South African Bantu develops malignant melanomas at least as frequently as the African European. Montpellier and Eisenbeth [31] commented on the frequency of melanomas among the Negroes, particularly female, in the Sudan and Cameroons and among the natives of Indochina.

There appears to be, on the other hand, a distinct discrepancy in the topographic distribution of melanomas between the two races. The trunk is the most frequent site of melanomas in whites, while the foot occupies this position in Negroes, in whom the less pigmented areas in general are the most common locations (soles of

* References 32, 34, 49, 52, 56, and 57.

† References 49, 58-63.

‡ References 65-67.

§ References 32, 56, and 72.

the feet, nail matrix, oral cavity, anus, and vulva).‖ The high incidence of melanoma in the foot of the Negro has been associated to the frequent chronic trauma and infections (crab yaws) related to barefoot living.¶ The evidence thus shows that the Negro is not peculiarly immune to malignant melanoma and exhibits a topographical distribution of such tumors which is not only related to his racially conditioned pigment distribution but also to the frequency and intensity with which exogenous factors act upon the most often affected parts of his body; i. e., the lower extremity.

5. *Cancers of Miscellaneous Sites.*—Several American investigators have claimed that the American Negro is less liable to develop cancer of the lung than the American white (ratio 1:2),# according to observations made in several southern states. However, a practically equal racial distribution of lung cancer was noted by observers reporting from northern and western states,* suggesting thereby that differences in the age distribution of the Negro population caused by their greater death rate for tuberculosis and other diseases may account for any regional differences in lung cancer mortality between whites and Negroes. Higginson found lung cancer not to be rare among the South African Bantus [55] treated in a Johannesburg hospital which draws its patient population from an urbanized area. The evidence on hand thus supports the view that race-connected differences in lung cancer mortality are not established. The existence of such relations is most unlikely because of the observation of similar differences among the inhabitants of two racially closely related countries, England and Norway, in regard to cancer of the larynx and lung. The mortality from cancer of the larynx in Norway was only one quarter of that found in England and deaths from cancer of the lungs were five times higher in England and Wales than in Norway in 1946.[83] This evidence again points to the operation of environmental local factors in bringing about geographical variations in cancer incidence. It is also for these reasons that recent assertions of a lower incidence of leukemia in American Negroes in comparison to that of American whites (ratio 1:3) should not be looked upon as an expression of a special racial resistance.†

Perhaps the most convincing demonstration of the fact that behind apparent or alleged race-connected carcinogenic factors are usually hidden actual environmental agents is presented by the demographic distribution of penile cancer. Penile cancers are practically nonexistent among Hebrews who for ritual reasons are circumcised during the first few days after birth; they are not common among Moslems who practice this religious procedure at the age of 5 to 6 years;‡ they are infrequent among the noncircumcised white population of the western world accustomed to proper personal hygiene measures, and they are unusually frequent among noncircumcised oriental populations (Chinese, Koreans, Indians, and Indonesians) § as well as excessively common among American Negroes in whom personal hygiene is often defective, phimosis is not surgically corrected, and venereal diseases are

‖ References 32, 56, 66, 67, 70, and 73-76.
¶ References 32, 66, and 70.
# References 77-80.
* References 81 and 82.
† References 84 and 85.
‡ References 86 and 99.
§ References 34, 36, 87-90.

not infrequent,‖ permitting thereby the prolonged and intense action of the decomposing smegma upon the skin of the glans and prepuce.¶ Since Jews and Moslems represent religious groups of complex racial composition, and because races of different habits and customs are involved, differences in penile cancer incidence cannot plausibly be explained on racial grounds.

A second example of the influence of exogenous carcinogenic agents on the apparently race-connected distribution of human cancers is to be found in the different frequency of oral cancers in Caucasians and the Malayan populations of India, Indonesia, and the Philippine Islands. Due to the practice of certain oral cancer-producing habits, such as betel quid chewing, khaini, and inversed smoking of cigars by certain parts of the population of these oriental countries, oral cancer is not only definitely more frequent in these population groups than among western peoples, but there is an inversed sex ratio present in some of the oriental countries (Philippines, Indonesia) favoring the female sex.#

Similar considerations against a predominantly genetic background of the demographic and geographic distribution of human cancers can be applied to cancers of the thyroid, which are frequent in endemic goiter areas, and to cancers of the esophagus,[100] which are common in China, Venezuela, and Panama.

B. Variations in Cancer Incidence in Different Regions of Individual Countries

While the consideration of the possible influence of race on cancer incidence is an important one when large geographical areas and population groups are analyzed, this complicating factor is of much less importance when the variations in different areas of smaller and less complex geographical and demographical units; i. e., population of individual countries, are studied. Any variations in the cancer incidence of different regions are less likely due to differences in the genetic composition of restricted population groups. At the same time, the importance of regional environmental influences as the real causes underlying such phenomena becomes more distinct.

From a map of the United States prepared by the American Cancer Society and showing cancer death rates for each state,[146] it appears that there is a range of rates extending from a minimum of 79 in Arkansas to a maximum of 150 in New York (Fig. 1). It is, moreover, evident that the majority of the states with high cancer death rates are located in the northeastern part, where they form a belt of highly industrialized regions. The high cancer death rate of California and the industrialized nature of several densely populated regions in this state follow this general pattern. The single exception is presented by the state of Nevada, in which a high cancer death rate is associated with mining and ranching as the chief occupational activities.

The apparent industrial influence on the incidence of human cancers becomes even more striking if 25 states selected for their definite occupational characteristics are grouped according to their main occupational activities and their lung cancer death rates for the years 1946 and 1948 (Table 8). The presence of the industrial-

‖ References 91-95.

¶ References 96 and 97.

References 34, 98, 101, and 102.

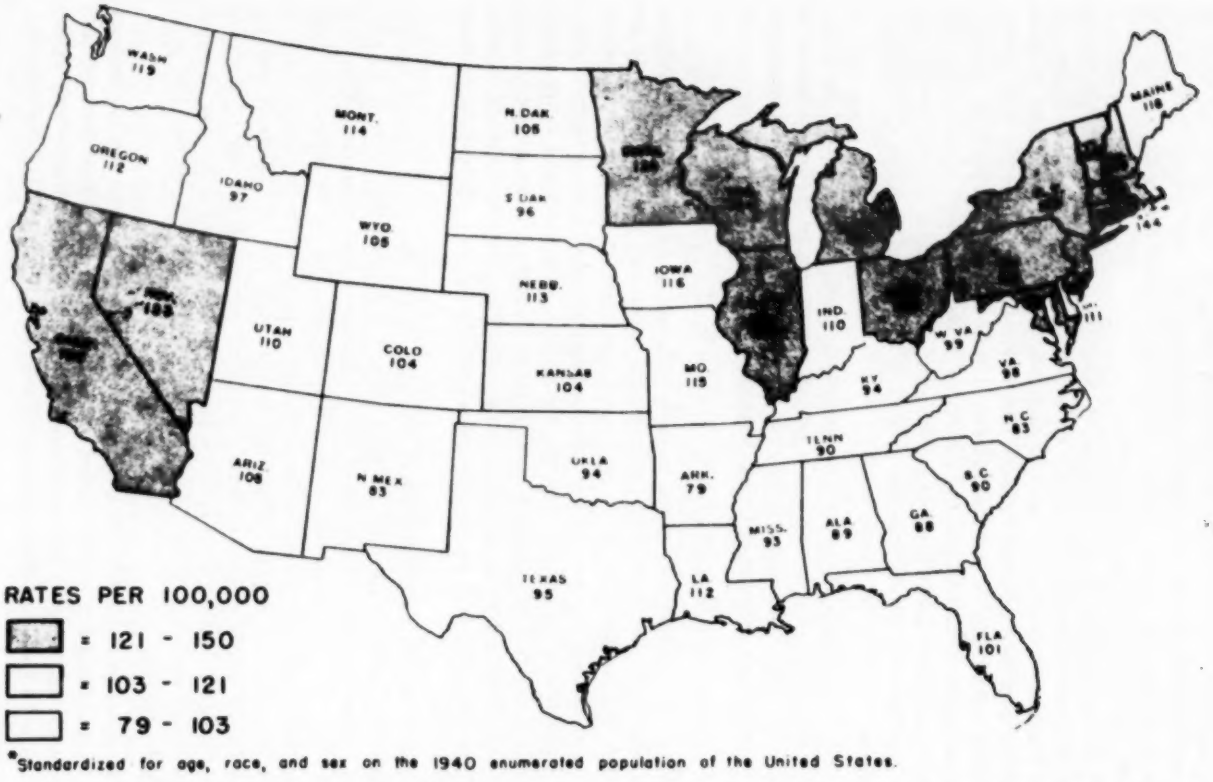

Fig. 1.—Cancer, all sites; death rates standardized for age, race, and sex,* 1939-1941. From Cancer Death Rates for Each State in the United States by Site, American Cancer Society, Inc., 1949.

ized states in the group with high lung cancer death rates and that of the agricultural states in the group with the low lung cancer death rate suggests a causal correlation between environmental factors and cancer incidence.

There is some evidence demonstrating the apparent effect of environmental factors upon the incidence of cancers for population groups inhabiting different areas in individual states. The map of Colorado (Fig. 2) provides an adequate illustration of the influence of industrial, agricultural, and mining activities upon the cancer death rates observed in three zones of Colorado where these activities predominate. An additional illustration of such relations is furnished by the distinct variations

TABLE 8.—*Lung Cancer Death Rates in Twenty-Five States of the United States in 1946 and 1948 Crude Death Rates per 100,000**

State	1946	1948
1. Industrialized States		
Connecticut	8.5	11.1
Illinois	8.1	8.2
Maryland	6.5	8.4
Massachusetts	10.4	10.2
Michigan	5.7	7.1
New Hampshire	7.4	10.1
New Jersey	9.7	9.7
New York	10.2	11.9
Ohio	6.0	7.3
Pennsylvania	6.7	8.4
Rhode Island	8.7	7.4
2. States with Regional Industrialization		
Florida	6.8	7.4
Louisiana	6.5	8.5
Missouri	7.3	9.4
Montana	10.0	8.8
Nebraska	5.7	8.0
3. Agricultural States		
Alabama	4.0	5.1
Arkansas	3.6	5.4
New Mexico	2.6	3.0
North Carolina	3.1	4.0
North Dakota	5.6	4.1
Oregon	4.1	4.4
South Carolina	3.6	3.7
Washington	5.1	4.2
Wyoming	4.9	3.9

* The death rates for the year 1946 were taken from The American Cancer Society, Inc., 1949, Cancer Death Rates for each State in the United States by Site, those for the year 1948 were produced by the National Office of Vital Statistics (Rigdon and Kirchoff).[147]

in lung cancer death rates in several counties in Montana in which special environmental and occupational factors have been operative for many decades and have thereby created a specific industrial background (Table 9).

In analyzing the cancer mortality statistics of the State of New Jersey [150] for the years 1940-1944, it is noted that the percent distribution of cancers of the urinary organs for males is listed as 10.3 in Salem County and as 16.6 in Salem City, while for the state as a whole it is only 6.5. Since this percentage distribution of cancers of the urinary organs is singularly high for Salem County and Salem City and since Salem County has a large industrial establishment with a known bladder cancer hazard it may be possible that this excessive regional rate of urinary cancers reflects a specific environmental hazard for the working population of this particular county.

It is likewise most unlikely that the marked variations in lung and larynx cancer morbidity established through recent surveys for eight metropolitan centers can be attributed to differences in the biologic susceptibility of their populations to cancers of the respiratory system (Table 10). The considerable differences in the morbidity rates of the populations as a whole and of both sexes and in the rates of progression between 1937 and 1947 suggest that basic differences and changes in the quantity and quality of some specific environmental factors are rather responsible for these local variations in lung and larynx cancer morbidity rates.

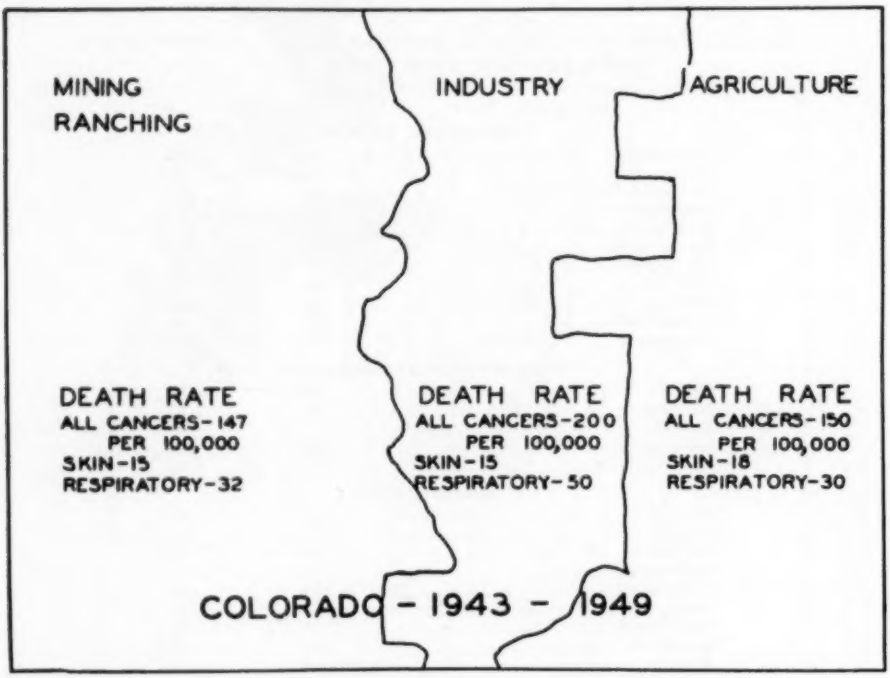

Fig. 2.—Specific cancer deaths in males.

TABLE 9.—*Lung Cancer Mortality in Several Counties of Montana, 1947-1948**

County and Total Population 1940	Major Industry	Number Lung Cancers			Total Cancer Deaths	Percent Lung Cancer		Annual Lung Cancer Death Rate/100,000	
		Male	Female	Total		Male	Female	Male	Female
Deer Lodge 13,627	Copper smelting	21	0	21	98	30.8	0.0	145.7	....
Silver Bow 53,207	Copper mining	27	2	29	259	22.6	1.5	48.6	3.9
Cascade 41,909	Copper mining; smelting	20	5	25	299	12.7	3.5	46.3	12.3
Gallatin 18,209	Agriculture	1	0	1	81	3.0	0.0	5.2	....

* The estimated crude death rate for lung cancer among white males in the entire United States in 1947 was 10.9 per 100,000 population.

The recent investigations of Stocks [149] in Great Britain on regional and local differences in cancer death rates have revealed the existence of similar marked variations in the death rates of cancers of various organs in different parts of England and Wales. Lung cancer death rates in 1921-1930 were highest in the industrialized regions of Manchester, Sheffield, Leeds, Nottingham, London, and Birmingham and very much lower in Stoke, Hull, Bristol, Portsmouth, and Leicester. The dispersion of lung cancer rates was very great; from 36 in Plymouth to 243 in Huddersfield. The only environmental factor which showed a significant correlation with death rates from lung cancer was the relative total annual amount of sunshine and smokiness of the atmosphere. In a somewhat more recent analysis of Fulton [150] similar positive relations were demonstrated between the incidence of lung cancer and the industrial or rural character of a number of communities studied

TABLE 10.—*Incidence of Respiratory Cancer, 1937 and 1947 Morbidity Rates for Eight Metropolitan Centers by Sex per 100,000 Population**

	Males			Females			Total		
Primary Site and City	1937	1947	Percent Increase	1937	1947	Percent Increase	1937	1947	Percent Increase
Bronchus and Lung									
Atlanta	5.0	13.4	168	1.0	5.0	400	2.9	8.9	207
New Orleans	13.1	39.1	198	2.8	4.2	50	7.6	20.8	174
Dallas	5.9	29.0	392	0.5	6.4	1,180	3.1	17.2	455
Birmingham	4.5	18.9	320	2.1	3.9	86	3.3	11.0	233
Denver	9.1	21.9	141	4.2	8.1	93	6.6	14.8	124
San Francisco	15.6	34.3	120	3.9	8.1	108	9.8	20.8	112
Chicago	13.3	29.5	122	4.3	7.0	63	8.8	18.0	105
Pittsburgh	9.7	26.1	169	4.9	5.5	12	7.3	15.6	114
Larynx									
Atlanta	1.4	4.0	186	0.3	0.3	...	0.9	2.0	122
New Orleans	11.3	14.9	32	0.4	1.0	150	5.6	7.6	36
Dallas	3.2	5.3	66	1.5	0.4	73	2.3	2.7	17
Birmingham	1.4	4.0	186	0.0	1.3	...	0.7	2.6	271
Denver	2.0	4.1	105	0.0	0.0	...	0.9	2.0	122
San Francisco	4.5	8.8	96	0.2	0.8	300	2.4	4.6	92
Chicago	6.7	7.0	4	0.4	0.6	50	3.5	3.7	6
Pittsburgh	4.4	8.0	82	0.4	0.8	100	2.4	4.4	83

* Biometrics Section, National Cancer Institute.

between 1944 and 1948. The regional incidence rates for lung cancer ranged from a low of 14 to a high of 28 for 100,000 population per annum. Similar variations in the incidence of cancers of other organs, such as the stomach, also were reported for England and Wales by Legon [151] and Cruickshank.[153]

The investigations of Dormans [154] uncovered the existence of regional variations in the incidence of cancer of the lung, esophagus, and rectum for different parts of Germany. Lung cancer rates were high for all cities located in northern Germany and the densely populated and highly industrialized areas of Saxony, Berlin, and the Rhein-Ruhr region, while they were low for the cities located in the Main area.

The observations attesting the presence of significant regional variations in different parts of individual countries furnishes additional circumstantial evidence that causation and incidence of human cancers appear to be correlated to a significant degree to environmental factors.

Recent investigations on the relative frequency of cancers, particularly cancers of the lung, among inhabitants of urban and rural areas have provided additional

important evidence in support of this concept. Studies on the demographic differentials in cancer mortality in urban and rural areas in the United States for the year 1940 have shown that the recorded mortality from cancer is higher in urban places than in rural areas, whether considered with regard to age, race, sex, marital status, or broad site group.[155] Even within urban centers, there is a uniformly higher cancer mortality in cities of 100,000 or more than in communities of lesser size. Although urban-rural differentials in cancer mortality may be particularly

TABLE 11.—*Mortality from Cancer in the North and South Sections of the Eastern and Central United States, by Population-Size Groups: Resident Age-Adjusted per 100,000 Population, 1940*

	Nonwhite Mortality by Population			White Mortality by Population		
Cancer	100,000 or More	2,500 to 100,000	Rural	100,000 or More	2,500 to 100,000	Rural
North	161.57	133.83	124.98	151.56	131.18	109.96
South	133.40	119.63	70.48	131.45	118.78	79.12

TABLE 12.—*Deaths from Cancer of the Respiratory System in Regions and Different Classes of Areas, 1940-1942 and 1943-1945*

		England and Wales	Greater London	East	South-west	Wales	County Borough	Other Urban Districts	Rural
Males all ages...	1940-1942	156	216	106	101	114	173	133	103
	1943-1945	188	249	131	127	143	199	159	126
Females all ages	1940-1942	41	51	35	33	38	42	37	36
	1943-1945	45	55	38	30	39	46	41	39

TABLE 13.—*Standard Mortality Ratios, England and Wales*

Divisions	E	WI	WII	NII	NIII	SE	SW	NI	MII	MI	NIV	London
Cancer of Larynx												
Male	79	80	89	90	92	93	97	98	98	105	108	147
Female	125	191	247	183	91	76	109	116	69	92	124	48
Cancer of the Lung												
Male	70	83	69	80	100	104	68	82	83	98	112	160
Female	75	69	69	95	99	107	76	86	88	96	102	150

affected by variations in the quality of diagnosis, medical care, and reporting of the disease, the observations made, it is concluded, may point up the influence of environmental factors on the disease. From a similar study by Pennell and Gover,[156] it appears that there is a general tendency for mortality rates of cancer to decrease with decrease in size of community and to be higher in corresponding population-size groups of the north section of the eastern and central United States than in those of the south section (Table 11).

In a statistical analysis of 2,407 admissions to the tumor clinic of a Veterans Administration Hospital, Schrek [51] noted that cancer patients from rural areas, representing 26.40% of all admissions, provided 32.8% of all cancers of the exposed skin, 44.3% of all cancers of the lip, but only 7% of the gliomas of the brain. Mills [161] found that respiratory cancer rates in different parts of Cincinnati varied in direct relation to the degree of air pollution present.

Kennaway [8] reported from England similar observations on the differential in cancer mortality between metropolitan and rural areas (Table 12).

Similar implications are contained in a more recent study of Phillips [157] on the standard mortality ratios (1946-1950) for cancer of the larynx and lungs (rates per million) in relation to density of population in 12 divisions in England and Wales (Table 13).

Kennaway and Kennaway [158] also concluded from studies of the incidence of cancer of the lung and larynx that in England and Wales the incidence of cancer of the lung in both sexes, and of cancer of the larynx in men, is higher in urban than in rural districts, while cancer of the larynx shows a reverse relationship (Table 14) for females.

Stocks [159] published recently an extension of his former studies on the endemiology of cancer of the lung in England and Wales covering the periods 1921 to 1939 and 1946 to 1949 and correlated the local incidence rates of lung cancer with the extent and density of built-up area as expressions of atmospheric pollution through

TABLE 14.—*Cancer of Lung and Larynx, England and Wales 1946-1949*

Type of Community	Lung Cancer Ratio-Number of Persons Producing One Death		Larynx Cancer Ratio-Number of Persons Producing One Death	
	Males	Females	Males	Females
Greater London	100	100	100	100
County Borough	129	137	135	55
Other urban districts	160	156	148	59
Rural districts	233	185	170	42

smoke from chimneys. He found that there was a general relationship between lung cancer mortality ratio and the above-mentioned two factors.

Groups of adjacent towns over 200,000 occupied dwellings	
London and suburban area	156
Birmingham	134
Manchester	159
Liverpool	162
Leeds	132
Sheffield with 124,000 occupied dwellings	135
Newcastle and Gateshead with 87,000 occupied dwellings	114
Aggregate of 6 towns with 50,000 to 85,000 dwellings	113
Aggregate of 3 towns with 40,000 to 50,000 dwellings	107
Aggregate of 12 towns with 30,000 to 40,000 dwellings	104
Aggregate of 13 towns with 20,000 to 30,000 dwellings	100
Aggregate of 29 towns with less than 20,000 dwellings	89

Stocks, moreover, demonstrated that the lung cancer mortality in various districts of Greater London apparently varied with the districts' positions in relation to the direction of the prevailing winds determining the smoke density in the various areas. In commenting on these observations, Stocks stated that they do not seem to square with the recently propounded concept that 90% of lung cancers are attributable to cigarette smoking. These observations and interpretations on the regional variations of lung cancer incidence thus parallel those of Hueper [160] advanced in connection with the marked variations in lung and larynx cancer morbidity established for eight metropolitan areas (Table 10) in the United States. It is, indeed, most unlikely that such marked discrepancies in the regional cancer incidence reflect fundamental variations of the smoking habits of the populations concerned. The

regional variations in lung and larynx cancer incidence as well as the irregular fashion in which the rates progressed during a 10-year interval between the two surveys of the eight metropolitan areas, present a pattern which conforms more closely to the action and changes of carcinogenic factors of occupational, industrial, or other nature.

It may finally be mentioned that Clemmesen [162] also noted that there was a distinct difference in lung cancer incidence between metropolitan areas, medium-sized and small towns, and rural areas in Denmark, that there was a lag of a full decade in the start of the rise in lung cancer incidence of Copenhagen and that of the rural districts, and that a difference in sex incidence in favor of males did not become obvious until after 1931. The Danish experience, repeating in a general way that in other countries during the past decades, thus also points to the importance of apparently complex environmental factors in causing lung cancer and changes in the incidence rate of the disease, affecting urban regions more than rural areas.

The environmental agents possibly responsible for such regional variations in the incidence of cancers in large population groups may be of general character, such as air, water, and soil pollutants, foodstuff contaminants, dietary deficiencies, endemic infections, universal habits and customs, and similar agents affecting population groups at large, or they may act upon only restricted parts or individual members of the population and then be associated with special industrial and occupational activities or cosmetics and medicinal agents.

While the evidence incriminating environmental agents of general character in the production of human cancer and in the determination of the regional incidence rates is only in part conclusive, it has grown during recent years in quantity and significance. European and American investigators * have established the fact that the exhaust fumes of gasoline and diesel engines contain appreciable amounts of carcinogenic hydrocarbons, that similar chemicals are demonstrable in the soot from domestic furnaces,[168] in the dust of asphalted roads,[167] and in the carbon black incorporated into automobile tires.† Carcinogenic material has been isolated from and identified in the particulate matter obtained from the air of English and American metropolitan areas.‡ The amount of such material increased more than fourfold on smog days.

It has been shown, moreover, that the general atmosphere contains, in varying degrees, two other carcinogenic agents; namely, arsenicals [171] and radioactive matter.[172] It stands to reason that such general background carcinogens will vary a great deal in amounts with local conditions, such as mining and smelting of arsenic, large-scale spraying of arsenical insecticides, or mining and processing of radioactive-material-containing ores, and similar special circumstances,§ which may also result in raising in some localities the normal level of these environmental carcinogenic contaminants in the water supply,‖ vegetables,[187] and fruits ¶ used for human consumption.

* References 164, 165, 177, and 180.
† References 169, 170, and 178.
‡ References 164-167 and 176.
§ References 190-193.
‖ References 183-186.
¶ References 188 and 189.

Carcinogenic hydrocarbons, for instance, have been demonstrated in barnacles growing on pilings in San Francisco Bay and are derived either from the creosote used in the impregnation of the wooden poles or from the ship fuel oil polluting the water of the harbor area.[181] There are in Texas several areas possessing drinking water supplies contaminated with petroleum. Since some of the native oils recently have been found to display mild carcinogenic properties in mice and rabbits,[194] such observations may not be entirely without significance in regard to environmental carcinogenesis. Increased amounts of arsenic have been obtained from the foliage of plants growing in the environment of copper smelters.

C. Variations of Cancer Incidence in Occupational and Socioeconomic Groups

While the circumstantial evidence relating cancer morbidity and mortality rates of general population groups to exposures to environmental cancer producing agents is, as a rule, rather indefinite as to the number and identity of individuals actually

Table 15.—*Standardized Mortality Ratio* at Ages 35-65 in Each Social Class from Cancer of Various Sites (Dicennial Supplements, 1921 and 1931)*

	Males									
	1921-1923					1930-1932				
Social Class	I	II	III	IV	V	I	II	III	IV	V
All sites	80	92	99	90	123	83	92	90	102	114
Lip	30	50	70	140	170	..	56	68	147	183
Tongue	48	73	95	100	165	78	69	98	110	143
Tonsil	25	88	94	106	163	(86)	76	93	97	147
Pharynx	89	78	100	160	161	(76)	83	90	87	130
Esophagus	76	91	104	88	130	74	87	98	94	130
Stomach	60	82	100	106	130	55	83	96	112	122
Upper alimentary canal	58	80	99	102	140	63	80	97	109	129
Lower alimentary canal	105	105	100	93	98	98	103	102	100	96
Larynx	72	96	98	96	135	60	81	98	90	143
Skin	63	73	100	120	150	(59)	75	95	116	133
Lung	100	109	97	79	124	107	96	101	91	112

* Registered percent of calculated deaths.

exposed and the types, degree, and duration of contacts with environmental agents sustained, data available in this respect on occupational groups and socioeconomic classes appear to be more explicit and informative.[103]

The demonstration of relations between cancer incidence by sites for various socioeconomic classes, which was attempted several decades ago by English and German investigators, and which led to the concept of "social cancer" * has recently received support from Denmark (Clemmesen and Nielsen).[108] It was shown in these investigations that, with few exceptions (lung and colon), the incidence of cancers of all sites for males increased with the decrease in the scale of the five socioeconomic classes. This relationship was somewhat less regular (no relation for colon, larynx, and lung) for females according to data given by Kennaway (Table 15).

The evidence on hand so far brings out the fact that there exist not only distinct differences in liability to cancer of various sites for different demographic, regional, urban, and rural population groups, but that within such groups there occur additional distinctions in attack rates of cancer related to socioeconomic subdivisions.

References 173-175.

* References 104-107 and 109.

In commenting on these observations, Kennaway and Kennaway [111] suggested that some of the differences observed might be attributable to differences in the degree of exposure of members of the various socioeconomic classes and sexes within such classes to more or less well-defined exogenous carcinogenic agents. They proposed, for instance, that the higher frequency of cancer of the scrotum in the lowest class might be due to greater uncleanliness and the associated more intense action of soot and grime. Ryle and Russell [112] concluded from their investigations on the socioeconomic distribution of skin cancer that the mortality also has a definite gradient with social groupings for the wives of workers, but for the males it would seem to have both a social and an occupational relationship and the latter is seemingly the more important. Kennaway and Kennaway suggested that the mortality gradient for cancer of the skin apparent for married women of Classes IV and V may be due to the employment of a proportion of married women mainly belonging to these two classes in the textile industry, where cutaneous contact with carcinogenic lubricating oils is known to exist. The absence of a social gradient for lung cancer was attributed by Kennaway [113] to the fact that owing to the mixing action of the wind there is less difference in the outdoor air breathed by different classes than in other social conditions such as food and cleanliness.

It is of interest to note that for cancers of the breast and ovary a reversed socioeconomic trend exists; i. e., the incidence rates for these cancers drop with the lowering of the socioeconomic status, although this order is not reversed for cancer of the uterus according to English observations (Kennaway,[106] Hewitt and Brooksbank),[107] while Clemmesen and Nielsen showed a clear tendency of cancer of the uterine cervix to occur more often in the poorer classes both among married and unmarried women.

Further elucidation of the relationship between the liability to cancer and the exposure to environmental agents is derived from the studies on the cancer incidence rates for members of individual occupational groups composing the different socioeconomic classes.† These initial investigations, which showed significant variations in the incidence rates of cancer of all sites as well as of special sites for different occupational groups, some being below, while others being several-fold above the respective incidence rates of the general population, have been confirmed and elaborated upon by more recent studies conducted in several industrialized countries (United States, Great Britain, Netherlands, and Denmark).

An analysis of the records of the Registrar-General (Great Britain) [111] in 1938 had shown that there was an excess of cancer of all sites in furnacemen, in rollers and their skilled assistants, in metal molders and die casters, in iron and steel foundry laborers, and in metal grinders; i. e., worker groups exposed to coal and oil, soot, tar fumes, lubricating oil mists, and various kinds of metal fumes and dust. In all four groups there was, moreover, an excess incidence of cancer of the lung, the highest occurring in metal molders and die casters. Turner and Grace,[119] in a study of the cancer mortality among males in certain Sheffield trades, likewise came to the conclusion that in six occupational groups (1. engineers, machinists, cutlers, etc.; 2. steel foundry and furnace workers, etc.; 3. grinders and sandblasters; 4. workers in precious and nonferrous metals; 5. workers in transport and com-

† References 105, 112, 114-118.

munications, and 6. coal miners) there was a significantly greater mortality from cancer than that experienced by clerks and business and professional men in the town.

In the first three groups, the excess cancer was definitely associated with the occupations. Cancer of the tonsil was particularly excessive among steel workers and cancer of the respiratory tract was marked among engineers, foundry workers, and grinders and in no other occupational group. The highest incidence of cancer of the alimentary tract was found in foundry and furnace workers—an observation suggesting that the carcinogenic factor might be the ingestion of hydrocarbons from fumes and smoke. Cancer of the pancreas, which is of surprisingly high frequency in Finland,[120] was excessive only in engineers and in workers in nonferrous metals. Cancer of the skin was significantly excessive in foundry workers and engineers.

From a very extensive investigation of Versluys [121] on cancer and occupation in the Netherlands, it is also quite evident that there is not an equal tendency among all people to be affected by cancer and that this shows itself in the distribution by organs typical for each trade. The total cancer mortality expressed as percentages of the expected one was increased in publicans, etc. (200%), butchers (161%), weavers (140%), fishermen (194%), green grocers (220%), cigar-manufacturers and makers (155%), tobacconists (121%), needlewomen (129%), and plumbers (225%) and was distinctly lower than the average of 100% in smiths (69%), firemen (61%), navvies (62%), female domestics (72%), and police (79%). The analysis showed, moreover, similar discrepancies in the relative incidence of cancers of various sites (excess and deficiency) for different trades and professions, as well as among the wives of those who carry on the different trades and women in female trades.

From a recent analysis of the deaths from cancer in males over 15 years old in Ohio during 1947 (41,526 deaths of all causes; 5,309 deaths of cancer of all sites) classified by occupation and cancer site, Mancuso obtained the data presented in Table 16.‡

This listing also provides a striking illustration of the fact that the liability to cancer of various sites possesses definite occupational relationships, which presumably are connected with occupation-specific carcinogenic exposures.

In addition to such broadly designed surveys on occupational relations to cancer in general and in various organs, there have become available several studies on specific cancer sites, providing supporting evidence. American and English investigators obtained evidence uniformly attesting the fact that outdoor workers, such as, especially, farmers, ranchers, fishermen, hunters, and house painters, have an excessive liability to cancer of the skin and lip.§

Ryle and Russell [112] found that there were not only distinct regional differences in skin cancer (England and Wales set at 100, Scotland 150, Northern Ireland 98, and Eire 230) as well as an increase in incidence with lowering of social classes I to V but also marked variations in relation to occupational groups (Group A [clean group; professions, clerks, typists, policemen] 70; Group B [heavy duty group; coal hewers, miners, iron ore workers, stone workers, textile workers, bricklayers, masons, etc.] 120; Group C [outdoor group; farmers, gardeners, builders,

‡ Mancuso, T.: Personal communication to the author.

§ References 122-129.

transport workers, dock workers, general laborers] 160; Group D [soot and grime group; furnacemen, firemen, stokers, boiler firemen, rollers]) 225. The conclusion is reached from this evidence that occupational relationships are more important than social class and that exposure to soot, grime, and sunlight is responsible for cancer of the skin of exposed sites. In similar investigations conducted by Atkin, Fenning, Heady, Kennaway, and Kennaway [130] on cancer of the skin and the lip

TABLE 16.—*Cancer Deaths Among Males 15 Years of Age and Over in Ohio Classified by Occupation and Cancer Site, 1947*

Industry	All Sites Percent of Total Deaths	Digestive Organs		Respiratory Organs		Genital Organs		Urinary Organs	
		Percent All Deaths	Percent All Cancer	Percent All Deaths	Percent All Cancer	Percent All Deaths	Percent All Cancer	Percent All Deaths	Percent All Cancer
Males 15 yr. and over	11.66	5.67	48.6	1.60	13.7	1.59	13.6	0.74	6.4
Service	12.78	6.25	48.9	1.76	13.8	1.76	13.8	0.81	6.4
Manufacturing industry	14.71	7.24	49.2	2.42	16.4	1.65	11.2	0.84	5.7
Retail and wholesale	13.58	6.28	46.2	2.26	16.6	1.85	13.6	0.71	5.2
Agriculture	12.00	6.40	53.3	0.82	6.8	1.99	16.6	0.82	6.8
Construction	13.31	6.03	45.3	2.12	16.0	1.78	13.4	0.69	5.2
Transportation and communication	13.83	6.71	48.5	2.91	21.0	0.98	7.1	0.90	6.5
Iron and steel industry	14.31	7.64	53.4	2.18	15.2	1.03	7.2	0.73	5.1
Electrical and machine	15.18	7.26	47.9	2.46	16.2	1.82	12.0	1.17	7.7
Nonferrous metal industry	15.32	7.80	50.9	3.22	21.0	1.21	7.9	1.48	9.6
Mining and quarrying	12.76	7.31	57.3	1.53	12.0	1.02	8.0	0.68	5.3
Stone, clay, and glass	10.09	4.13	41.3	0.66	6.5	1.32	12.0	0.88	8.7
Rubber and plastic industry	14.54	7.01	48.2	2.34	16.1	2.06	14.3	0.52	3.6
Others and unknown	11.46	5.54	48.3	1.29	11.3	1.93	16.9	0.81	7.1

Industry	Lip; Oral Cavity; Pharynx		Skin		Brain and Central Nervous System	
	Percent All Deaths	Percent All Cancer	Percent All Deaths	Percent All Cancer	Percent All Deaths	Percent All Cancer
Males 15 yr. and over	0.60	4.7	0.32	2.5	0.21	1.6
Service and manufacturing industry	0.58	3.9	0.33	2.2	0.40	2.8
Retail and wholesale	0.71	5.2	0.24	1.8	0.28	2.1
Agriculture	0.32	2.7	0.57	4.8	0.09	0.8
Construction	0.69	5.2	0.48	3.6	0.30	2.3
Transportation and communication	0.76	5.5	0.18	1.3	0.13	1.0
Iron and steel industry	1.15	8.0	0.06	0.4	0.30	2.1
Electrical and machine	0.52	3.4	0.13	0.8	0.52	3.4
Nonferrous metal industry	0.40	2.6	0.13	0.9	0.00	0.0
Mining and quarrying	0.17	1.3	0.85	6.7	0.17	1.3
Stone, clay, and glass	1.10	10.9	0.44	4.3	0.00	0.0
Rubber and plastic industry	0.26	1.8	0.00	0.0	1.30	8.9
Others and unknown	0.59	5.2	0.29	2.6	0.11	1.0

in males engaged in agriculture, mining, and professional occupations in England and Wales from 1911 to 1944, it is noted that the mortality from cancer of the skin and the lip is highest among agricultural workers and lowest in the professional classes, while the miners take an intermediate position. The incidence in coal miners was of special interest, since it was lower than that of the general population. Only one occupation in social class I (civil engineers and surveyors) showed a mortality from cancer of the skin higher than that of the general population. The difference between the professional classes, on the one hand, and the miners and agricultural workers, on the other hand, was much greater in the case of cancer of the lip than in that of cancer of the skin. While the special liability of outdoor workers

to cancer of the skin and lip is generally attributed to exposure to sunlight, the data on their topographical and sex distribution obtained in this survey indicated that other factors are involved also.

The validity of this assertion is attested by the studies of Cruickshank and Squire [131] and Cruickshank and Gourevitch [132] on skin cancer among members of the engineering industry and on the occupational liability to cancer of the hand and forearm. It was shown that a risk of skin cancer may occur in the engineering industry as a result of exposure to mineral oils and that a significantly large proportion of skin cancers involving the hand and forearm occurs among "metal workers," probably caused by an exposure to mineral oil. This occupational group as well as cotton mule spinners; chimney sweeps; gas, tar, pitch, and creosote workers; fishermen; boatmen; bargemen; boat builders, and fishing-net makers, as well as engine and crane drivers, firemen, and cleaners, were found to have an excessive liability to cancer of the scrotum due to an occupational exposure to coal tar, pitch, creosote, and lubricating oils.[111] The liability to scrotal cancer appeared to be higher, moreover, among town dwellers exposed to soot than among residents of rural areas.

The numerous observations which during recent years have been made on the environmental and occupational relations to cancer of the lung are equally instructive. Kennaway and Kennaway [133] thus reported that the incidence of cancer of the lung in coal miners is less than that in the general population and is similar to that of agricultural workers. McConnell, Gordon, and Jones [134] contended that they were unable to establish any relations of occupational factors to the occurrence of lung cancer in a series of cases collected in the Liverpool region and tested for occupational exposures of three types: (*A*) cement and road dust; railway, coal, and flue dust; metal fumes and dust; oil; tar; (*B*) wood dust; cotton dust; flour; tobacco; vegetable dust; (*C*) printer's ink and paper dust; paint; chemicals. There was, according to the investigators, no significant difference between the cancer and control groups with regard to occupational factors, although for Group A, containing a number of known and established respiratory carcinogens, there were 63.8% cancer patients against 50.0% control patients. The failure of McConnell and co-workers to demonstrate occupational relations is evidently due to an unsuitable selection and arrangement of factors for the testing of statistical correlations.

This conclusion is supported by the results of an analysis which Heuper [169] recently made of a larger number of old and recent epidemiologic studies on this subject, from which he compiled the pertinent facts presented in Table 17.‖

Kennaway and Kennaway [133] noted that a group of open air occupations, where there is exposure to the dust of roads, has ratios above 100 for cancer of the lung and larynx, with the exception that motor drivers have a normal liability to cancer of the larynx, which is excessive among metal grinders, cardroom jobbers, tobacco manufacturers, gas works managers, lithographic and process engravers, and blast-furnace men.

An etiologically significant role of metal dusts and fumes and coal and oil soot in the production of lung cancer is also suggested by the recent investigations of Breslow,[137] who noted in an occupational analysis of 408 cancers of the lung an excessive frequency of welders, cranemen, derrickmen, firemen, miners of non-

‖ References 138-140.

ferrous metal ores, oil drillers, and tool dressers among the occupations listed for the cancer cases in comparison with the frequency among an equal-sized control group.

Another example indicating a possible relation between an occupational exposure to coal and fuel oil soot and an excessive liability to lung cancer is presented by an apparently increased frequency of this cancer among operating railroad workers in comparison to nonoperating railroad workers (Table 18). Since the employment ratio of operating railroad workers to nonoperating ones is about 1:4, it is evident that about 75% of the lung cancers among railroad employees of the two companies studied occurred among the operating group, representing only 25% of the total number of employees.

TABLE 17.—*Occupational Groups with An Excessive Lung Cancer Incidence*

Occupational Group	Investigator
Metal workers, welders, metal grinders and polishers, wire makers, tool and die makers, foundry workers, metal molders, lathe workers, etc.	Borst; Kennaway and Kennaway; Turner and Grace; Mueller; Dublin and Vane; Wynder and Graham; McLaughlin
Cigar manufacturers and tobacconists	Seyfarth; Borst; Kennaway and Kennaway; Enger; Versluys; Brinkmann
Engineers, mechanics, machinists, plumbers, etc.	BECC* 1944 and 1952; Gillespie; Turner and Grace; Mueller; Wynder and Graham
Painters, decorators	BECC* 1944; Mueller; Dublin and Vane; Fulton; Wynder and Graham
Tar workers, road workers, asphalters, paviours, stokers, patent fuel workers, furnace men, foundry laborers, rollers, etc.	Kennaway and Kennaway; Fulton; BECC* 1952; Registrar-General 1938; McLaughlin

* Report of British Empire Cancer Campaign.

TABLE 18.—*Lung Cancer Frequency Among Operating and Nonoperating Railroad Workers*

Railroad	Period	Total No. Lung Cancers	Lung Cancers Operating Railroad Workers		Lung Cancers Nonoperating Railroad Workers		Lung Cancers Undetermined Railroad Workers	
			No.	Percent	No.	Percent	No.	Percent
A	1940-1950	29	24	83	5	17	..	..
B	1939-1949	104	59	57	15	14	30	29

Additional evidence suggesting the existence of relations between an excessive liability to cancer of the lung and occupational factors was uncovered when the death records of the International Photo-Engravers' Union were evaluated. Among a total of 1,520 deaths observed during 1940 to 1949 there were 165 deaths from cancer of all sites, including 45 pulmonary cancers. The estimated death rate from cancer of all sites for the population of the United States having the same age composition as the union during 1946 to 1950 was 133, while the actual number of deaths among members of the union for the same period stood at 94. However, there were 27 lung cancer deaths in this group (29%) instead of the estimated 20 (15%). While these observations are in general agreement with those made on photoengravers holding policies with the Metropolitan Life Insurance Company, showing 33.3% of deaths from cancer of the lungs among deaths from all forms of cancer, a recent survey of 684 living photoengravers by chest x-ray examinations failed to yield any pulmonary neoplasms.¶

¶ References 141 and 142.

The total evidence presented on the relationship of socioeconomic and occupational groups to relative liability to cancer not only leaves little doubt about the actuality and general significance of such connections but also suggests strongly that such correlations are of importance for cancers involving the great majority of organs and tissues.

D. Sex Distribution of Cancers and Environmental Factors

Although the evidence supporting the action of environmental factors in the production and incidence of cancers is much more extensive for males than for females, the data on hand, nevertheless, attest that they operate also in connection with cancers in females. A great deal of reliable data demonstrate that there is a marked incidence differential favoring the male for cancers of the skin, lip, oral cavity, larynx, lung, stomach, esophagus, liver, and bladder, while the sex ratio is in favor of the female for cancers of the gall bladder and thyroid. Such observations per se may be interpreted as showing that differences in exposure conditions are responsible for the sex ratios noted for at least cancers of some of the sites mentioned (skin, oral cavity, lip, lung, larynx, and bladder). More convincing, however, is the fact that these sex ratios are variable for different regions, population groups, and races as well as for different periods and may even show under certain circumstances an inversed character. The sex ratios, therefore, evidently are not sex-connected but exposure-related.

The male-female sex ratio of lung cancer, for instance, covers a range from 1 : 1 to 1 : 20 as given by different investigators. In fact, the ratio has even changed considerably in several countries during the past 25 years. While the male-female ratio in Denmark in 1931 stood at 5 : 4, the gap between the sexes in lung cancer incidence had widened by 1945 to 3 : 1.[143] Similar observations were recently reported by Kreyberg from Norway,[144] who found a sex ratio of approximately 1 : 1 between 1929 to 1936 and 2 : 1 in 1949. Corresponding increases in the sex differential of lung cancer were recorded from England [145] and the United States.[187]

The sex ratio of cancer of the larynx revealed remarkable regional differences in England and Wales (Table 13) [187] as well as in the United States (Table 10), the latter showing additional variations for the individual metropolitan areas in the two surveys made at an interval of 10 years (1937 and 1947). The sex ratio of oral cancers existing for white women is reversed for Filipino women indulging in betel quid chewing. The sex ratio of skin cancer is markedly different for whites and Negroes. If these observations are correlated with those made in connection with sex ratios found with occupational cancers, where exposure factors are clearly the determining influence, it becomes evident that the existence of sex differentials supports the concept of the significance of environmental factors in the genesis of human cancer.

E. The Changing Cancer Incidence Rates

To the evidence of general character cited in confirmation of this dogma, there may be added the increasing amount of information concerning the occurrence of distinct changes in the morbidity and mortality rates of cancers of several sites.# These changes have been most spectacular for cancers of the lung, which have shown a consistent increase in frequency starting some time after the turn of the century

References 195 and 196.

and gaining momentum during recent decades. The rise in lung cancer exhibited distinct irregularities as to its time of onset, its progression rate, and its sex differential in different localities and regions. It has by now been observed in all industrialized countries.[160] Since such an epidemiological behaviour cannot justly be attributed to the development of fundamental alterations in the biologic properties of the various populations affected, it may be assumed that the rapid and marked accentuation of the modern industrial environment preceding and accompanying this rise in lung cancers is most probably in some way connected with it.

Males have had a decidedly and increasingly larger share in this development than females. Although many investigators have clung to the illusion that the manifold increase of lung cancer within two to three decades was for various reasons merely an apparent one, it has become practically certain that the phenomenon is real and serious.* A rapid increase of cancer of the lung has occurred during the past 30 years even among the English coal miners, although they have a relatively low incidence of the disease.[133] Whether or not these alterations in lung cancer frequency have been brought about by carcinogenic factors in our general environment (air pollution), by a number of specific industrial and occupational factors involving larger parts of the general population, or by the enormous increase in the cigarette smoking habit during the past five decades is at present a subject of lively controversy.† The total information available at the present time makes it most unlikely that one single factor has been responsible for the major portion of this rise. Such a concept may fit well one facet of the problem. It is scarcely in agreement with the general pattern presented by the rest of the factual evidence, which suggests that apparently several, or many, factors connected with modern industrial activities and present-day living are causally involved in this development and that these factors have operated in different communities, regions, and population groups in various quantitative and qualitative combinations and started to act at different times.

Less striking, but equally alarming, is the increase observed in the mortality rate from leukemia during the past three decades. In the United States, the crude mortality rate from leukemia has risen from 1.0 per 100,000 of population in 1900 to 4.3 per 100,000 in 1944.‡ This change is not accounted for by differences in the age distribution of the population during this period. Similar statements have been advanced by Shimkin,§ Sturgis,[202] and Pizzolato [85] in this country. The increase was apparently more marked among nonwhites than among whites.[197] English and German investigators also reported a rise in the leukemia rate during recent years.‖ The evidence on hand suggests that there has been a twofold increase in the over-all incidence of leukemia in Scotland between 1938 and 1951. The reasons for this development are totally obscure, and relatively little attention has so far been paid to it, although leukemia is an invariably fatal disease. Apart from the claim that better diagnoses during recent years are responsible for this development, occupation, diet, infectious diseases, medicinal agents, and trauma have been con-

* References 157 and 160.
† References 160 and 198.
‡ References 199 and 203.
§ References 200 and 201.
‖ References 204-207.

sidered in this respect, but no significant or even suggestive relations have as yet been established. The pattern of events, as well as the use of many chemicals in industry and medicine eliciting toxic and allergic hypoplastic and hyperplastic (ambivalent) reactions of the hematopoietic tissue point to the effects of newly introduced environmental agents as the causes of this phenomenon. The relatively high frequency of leukemia in infants and young children does not militate against this supposition because of the possibility of a transplacental carcinogenic stimulation of the hematopoietic tissue of the fetus by chemicals entering the maternal organism or by transfer of such agents to the infant with the maternal milk.

IV. ENVIRONMENTAL CANCERS AND CARCINOGENS

The existence of significant relations between environmental agents and the development of cancer, which is made most probable by the various types of general evidence cited above, is definitely and convincingly established by the demonstration of the different environmental cancers produced by well-defined physical and chemical agents and not infrequently associated with the appearance of characteristic functional and anatomic symptomatic manifestations of pericancerous and precancerous nature. In their best-developed forms they present disease entities as clearly circumscribed and definitive in etiologic and symptomatic respects as many infectious diseases. They may display then, like some of the infectious diseases, several developmental phases, which, however, because of their delayed appearance and long duration, are less readily recognizable than those seen with infectious disorders.

Environmental cancers have, similar to the latter, characteristic epidemiologic patterns of spread, while their attack rates vary with the inherent potency of the environmental carcinogenic agent and the intensity, duration, and generality of contact ¶ with a population composed of constitutionally heterogeneous individuals. Climatic and seasonal influences or working conditions may modify the epidemiologic pattern by increasing or decreasing the degree of exposure through altering the permeability of the skin to the carcinogenic agent or the depth and frequency of respiratory movements, although such effects upon the individual susceptibility and the general incidence rate may not become apparent until many years later, in contrast to their appearance within days, weeks, or months where infectious diseases are concerned. The preparatory developmental and symptomatic events in environmental cancers proceed in "slow motion."

Since a detailed account on occupational and environmental cancers was rendered some 10 years ago,[209] it may suffice to present at this occasion only the high-lights of knowledge newly acquired since then and to call attention to some of the developments which may be anticipated and watched for during the coming years.

A. Inorganic Chemicals

1. *Arsenic.*—Although there is still no reliable evidence in experimental animals on the carcinogenicity of arsenicals, the past two decades have brought a good deal of additional observations attesting the fact that arsenicals are at least conditional carcinogens to man. Neubauer [215] in 1947 culled 143 medicinal arsenic cancers of the skin and 24 occupational cancers from the world literature. These data, however,

¶ References 208-214.

did not include the environmental arsenic cancers formerly observed in the Reichenstein region, where the drinking water supply had been contaminated with arsenic from slag heaps of mines, and the more recently reported endemic arsenic cancers attributable to the same source in Cordoba, Argentina. In their last report, Arguello and Tello [216] have collected a total of 148 cases of skin cancer of which 90 showed cutaneous manifestations of chronic arsenicism. The topographical distribution of the cancerous lesions observed was characteristic for an arsenic causation because only 37 of the cancers affected the head, lip, and neck, while 111 involved the trunk and extremities. In solar cancer of the skin, also frequently observed in Argentina according to Roffo,[217] about 90% of the neoplasms are found on the head and neck.

Several cases of industrial arsenic cancers of the skin have been placed on record since 1947 from various countries, particularly as the result of manufacturing and using arsenical insecticides (five cases).# None of them originated in the United States, although industrial arsenicals have been produced and consumed in this country for many years to a much higher degree than in any other country. That this lack of information on industrial arsenic cancer is not indicative of a special immunity of our countrymen against the carcinogenic action of arsenicals, however, is strikingly demonstrated by recent reports on medicinal arsenic cancers, totaling 37 cases.* Since 27 of the 37 cases were reported from one institution and 8 from another, it stands to reason that there must occur many more arsenic cancers which go unnoticed because of insufficient arsenic-consciousness of the medical profession.

A great deal of attention has recently been aroused by the claim that the inhalation of arsenic dust and fumes might give rise to cancer of the lung. Neubauer [215] already has wondered whether the occurence of five cancers of the lung among 24 individuals suffering from occupational arsenic dermatoses or cancers in contrast to only two cases of lung cancer among 143 cases of medicinal arsenic cancer of the skin was a mere coincidence or whether, under occupational conditions, the irritation of the respiratory tract by arsenical dust was responsible for the phenomenon. There have been two additional cases of lung cancer among 10 sheep dip workers, according to Henry,[225] and five lung cancers among the chemical workers of a sheep dip factory [226] in England.[218] It is not quite certain, because of unsatisfactory statistical data, whether the six cases of cancer of the lung observed by Snegireff and Lombard [227] among American copper smelter workers can be included in this group of arsenic cancers of the respiratory system.

Additional interest in the possibility of a carcinogenic action of arsenic on the lungs is related to the fact that arsenic is contained in the suspended matter of town air [228] as well as in the tobacco smoke inhaled.[229] From the evidence available on this point, and discussed in detail elsewhere,[160] it is not likely that the inhalation of arsenicals from these two sources plays a major role in the production and increased incidence of cancer of the lung.

2. *Asbestos.*—The evidence indicating the existence of causal relations between asbestosis and cancer of the lung has received additional support during recent years from several countries (United States, Canada, Great Britain, Germany, and France).† There is, at present, a total of 60 cases of asbestos cancer of the lung on

References 218-220.

* References 221-224.

† References 235-253.

record. Ten of these cases were reported from this country. The male-female ratio is 2.5 : 1, demonstrating a marked shift toward the female side from the usual sex ratio of about 10 : 1 and providing, thereby, an illustration of the thesis that with an equalization of carcinogenic exposure for the two sexes there results a trend toward equalization of liability to cancer. The incidence rate of cancer of the lung in cases of asbestosis seen at autopsy in Great Britain and Germany stands at about 15%,‡ which agrees with the percentage figure observed in a larger American series. Since several attempts have been made to discredit the evidence incriminating the causal relation between an effective exposure to asbestos, evidenced by the development of asbestosis of the lung, and cancer of this organ by citing clinical observations made on asbestos workers at large, it should be pointed out that such methods are unreliable in establishing the existence of cancer of the lung in the presence of asbestosis and produce by the inclusion of many individuals without asbestosis, an unscientific dilution of the pertinent and valid evidence only procurable by post-mortem studies.

It appears from the data available that asbestos corns, which are granulomatous warty formations developing around asbestos needles impacted in the skin, do not give rise to cancers.§

3. *Chromium.*—Recent investigations of American chromate manufacturers have confirmed the previous German observations‖ that the members of this occupational group have a highly excessive liability to cancer of the lung, demonstrable by both mortality and morbidity studies.¶ A survey of an English chromate plant [265] with 786 workers, on the other hand, gave inconclusive results as to the existence of a special lung cancer hazard. While some recent German observations suggest that chrome pigment workers also share in the lung cancer hazard,[266] there is at present no evidence available concerning the existence of such a liability for other workers having occupational contact with chromium, chromium alloys, powders, and sprays, as well as with chromic acid and chromium compounds. In view of the fact that the epidemiologic information does not give definite evidence as to the actual nature of the carcinogenic agent and because of the inconclusive results of producing cancer in experimental animals by various chromium compounds and different routes of administration of these chemicals there is an urgent need for additional epidemiologic studies of other worker groups exposed to chromium in its various forms (stainless steel manufacturers, chromium metal sprayers, chromium powder metallurgists, chromium platers, dye manufacturers, chromium pigment workers, welders, zinc chromate sprayers, etc.).# Such an extension of epidemiologic surveys is indicated because Schinz and Uehlinger [267] found osteogenic sarcomas in two rabbits three years after an intraosseous implantation of metallic chromium.

The total number of chromate cancers of the lung stands at about 125, of which approximately one-half were observed in American plants. Since the crude death rate for cancer of the lung was 25 times the normal and the morbidity rate about 42 times the normal, it may be concluded that the carcinogenic agent causing cancer of the lung among chromate workers is apparently a carcinogen of high potency.

‡ References 237 and 240.
§ References 254-258.
‖ References 259 and 271.
¶ References 260-264.
# References 268-270.

4. *Nickel.*—According to the report of the Chief Inspector of Factories, England and Wales, there were observed between 1923 and 1948 among the workers of the copper-nickel matte refineries at Clydach, England, a total of 47 cancers of the nasal cavity and paranasal sinuses and 82 cancers of the lung, occurring in an adult male population of approximately 14,000.[272] Three additional cases of lung cancer were recently reported from a Norwegian nickel refinery.[273] According to Goldblatt,[274] no cases of respiratory cancer have come to observation within a 10-year-period in German nickel refineries. Cancers of the nose were most often found among workers engaged in the calcining and roasting operation, where the workers are exposed to the inhalation of a coarse alkaline nickel dust. They were less often seen among workers employed in the nickel carbonyl operation. Cancers attributed to occupational exposures to nickel have not as yet been reported from American sources, although the United States is a prominent processor and industrial consumer of nickel in its various forms, including nickel carbonyl (catalyst in the production of acrylic acid esters).[275]

The successful production of cancers in rats following the parenteral inplantation of powdered metallic nickel indicates that it is nickel as such which is carcinogenic.[276] Apart from the industrial importance of these findings, they may have some significance in possibly explaining the occasional occurrence of cancers around metallic foreign bodies, such as bullets, embedded in tissues.[277]

These observations and others relating to experimental cancers, to be mentioned later, provide a rather sound basis for the concept of "metal cancers" first proposed by Schinz. They should provide, moreover, an indication for studying, by epidemiologic methods, all types of "metal workers" for special cancer hazards and for subjecting individual cancer cases to a scrutinizing analysis of their occupational and environmental background for metal exposures sustained in the past. Such investigations may well include persons who have received, for medicinal reasons, implants of metal and metal alloys.

(To be Continued)

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News and Comment

American Board of Pathology Changes in Requirements for Certification in Special Fields of Pathology.—The following changes in copy were received too late for inclusion in the statement published in the September, 1954, issue of the ARCHIVES: The words "Clinical Chemistry, Clinical Microbiology, Hematology, and Neuropathology" should follow the word "Pathology" in the heading of this notice; the effective date should be July 1, 1954, instead of July 1, 1955.

Dr. Vorwald Goes to Wayne University.—Dr. Arthur J. Vorwald, former director of the Edward L. Trudeau Foundation laboratories at Saranac Lake, N. Y., recently became head of the newly created department of industrial medicine in Wayne University College of Medicine, Detroit. A Naval captain in World War II, Dr. Vorwald served as medical attaché in the American embassy in London and was director of the medical division of the Office of Naval Research. He formerly taught at the University of Rochester and at New York University.

Dr. Schmeisser Becomes Emeritus Professor.—Dr. Harry C. Schmeisser, who has retired after 33 years as professor of pathology at the University of Tennessee Medical Units and attendant to John Gaston Hospital, Memphis, has been appointed professor emeritus of pathology and consultant to the city of Memphis hospitals. Dr. Schmeisser joined the staff of the university in 1921 as professor of pathology and bacteriology, chief of the division, and pathologist and bacteriologist in chief to John Gaston Hospital.

ANNOUNCEMENT

Symposium.—A Symposium on the Nutritional Aspects of Blood Formation will be held at the University of Cincinnati on Friday, Oct. 22, 1954. This Symposium is being made possible by support from The National Vitamin Foundation and will feature reports and discussions on the stated subject by a selected group of specialists in the field.

The meetings of the Symposium will begin at 9:15 a. m. in the Auditorium, College of Medicine, Eden and Bethesda Aves., Cincinnati, and will continue through 5:00 p. m. The morning session will be under the chairmanship of Dr. Richard W. Vilter, Associate Professor of Medicine, College of Medicine, University of Cincinnati, and the afternoon session will be under the chairmanship of Dr. M. A. Blankenhorn, Professor of Medicine, University of Cincinnati, and Director of the Department of Internal Medicine, Cincinnati General Hospital.

The Symposium is open to all interested physicians and scientists.

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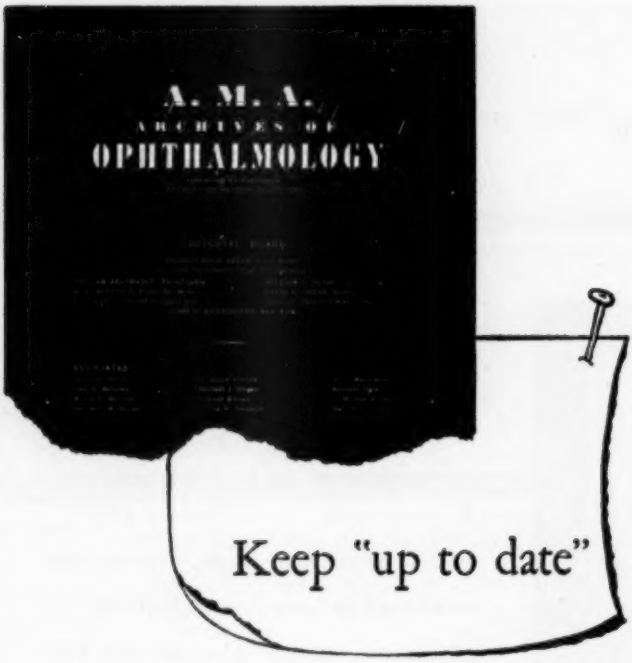

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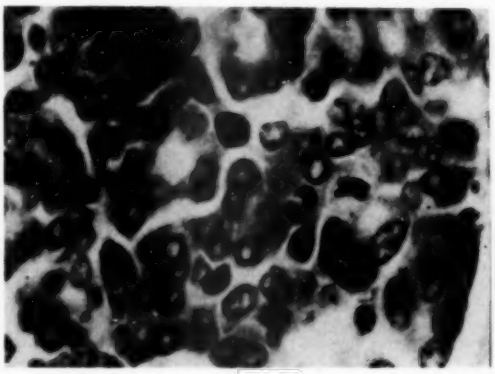

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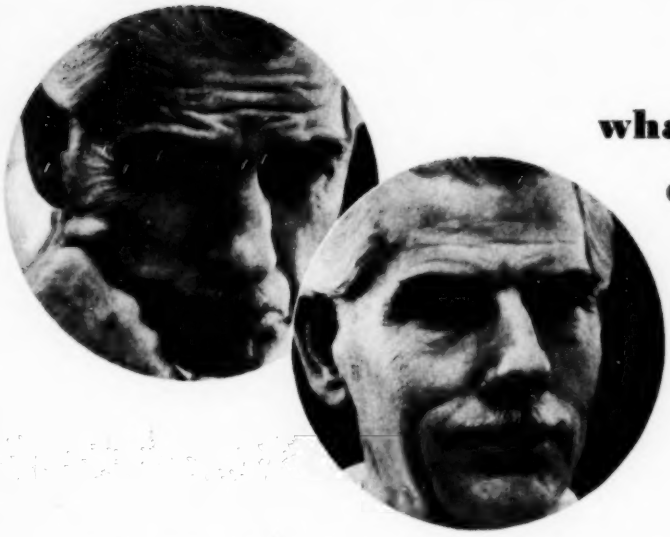

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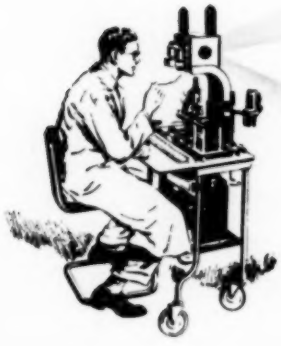

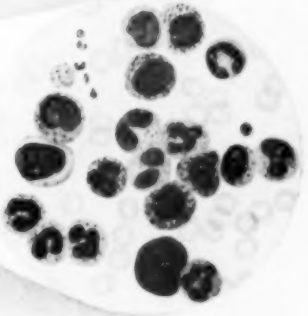

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